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Ministry of Health & Family Welfare

Pharmacopoeia of India

(The Indian Pharmacopoeia)

Volume – II
(Q – Z & Appendices)

Third Edition

सत्यमेव जयते

PUBLISHED BY THE CONTROLLER OF PUBLICATIONS, DELHI

1985

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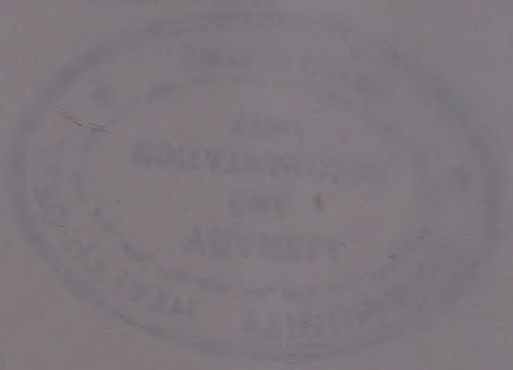


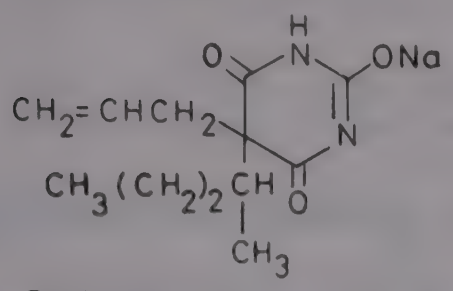
Pharmacopoeia of India
(The Indian Pharmacopoeia)

Volume – II
(Q – Z & Appendices)

Monographs (Contd.)

Quinalbarbitone Sodium

Secobarbital Sodium; Soluble Quinalbarbitone; Quinalbarbital Sodium



$C_{12}H_{17}N_2NaO_3$ Mol. Wt. 260.27

Category : Hypnotic; Sedative.

Dose : 0.1 to 0.2 g.

Description : White hygroscopic powder; odour less; taste, bitter.

Solubility : Freely soluble in *water* and in *alcohol*; practically insoluble in *chloroform* and in *solvent ether*.

Standards : Quinalbarbitone Sodium is sodium 5-allyl-5-(1-methylbutyl) barbiturate. It contains not less than 98.5 per cent and not more than the equivalent of 102.0 per cent of $C_{12}H_{17}N_2NaO_3$, calculated with reference to the dried substance.

Identification : (A) Dissolve 0.3 g in 10 ml of *water*, and divide the solution into two portions. To one portion add 1 ml of *mercuric chloride solution*; a white precipitate is produced which is soluble in excess of *dilute ammonia solution*. To the other portion add 5 ml of *silver nitrate solution*; a white precipitate is produced which is soluble in excess of *dilute ammonia solution*.

(B) To 0.5 g add 5 ml of *sodium carbonate solution* and 10 ml of *nitrobenzyl chloride solution*. Heat for 30 minutes on a water-bath under reflux and allow to stand for one hour. Filter, wash the precipitate successively with 10 ml of *dilute sodium hydroxide solution* and 50 ml of *water*; recrystallise from a mixture of equal volumes of *chloroform* and *alcohol* and dry at 100° to 105°. The crystals melt at about 156°, Appendix 5.11.

(C) Ignite; the residue gives the reactions of *sodium*, Appendix 3.1.

(D) Dissolve 0.1 g in 5 ml of *water* and add 5 drops of *0.1N potassium permanganate*. The colour of the permanganate is discharged immediately.

pH : Not more than 11.0, determined in a 10.0 per cent w/v solution, Appendix 5.10.

Neutral and basic substances : Carry out the test described under Amylobarbitone. The residue weighs not more than 3 mg.

Heavy metals : Not more than 30 parts per million, determined by Method C on 0.67 g, dissolved in a mixture of 5 ml of *N sodium hydroxide* and 20 ml of *water*, Appendix 3.2.4.

Free quinalbarbitone : Complies with the test for **Free amylobarbitone** described under Amylobarbitone Sodium.

Loss on drying : Not more than 5.0 per cent determined on 0.5 g by drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the **Assay** described under Amylobarbitone Sodium. Each ml of *0.1N alcoholic sodium hydroxide* is equivalent to 0.02603 g of $C_{12}H_{17}N_2NaO_3$.

Storage : Store in tightly-closed containers, protected from moisture.

Quinalbarbitone Tablets

Quinalbarbitone Sodium Tablets; Secobarbital Sodium Tablets

Category : Hypnotic; Sedative.

Dose : Quinalbarbitone Sodium, 0.1 to 0.2 g.

Usual strength : 0.1 g.

Standards : Quinalbarbitone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of Quinalbarbitone Sodium, $C_{12}H_{17}N_2NaO_3$. The tablets are coated.

Identification : (A) Shake a quantity of the powdered tablets equivalent to about 0.5 g of Quinalbarbitone Sodium with 10 ml of *water* and filter. The filtrate complies with **Identification** test (B) described under Quinalbarbitone Sodium.

(B) Triturate a quantity of the powdered tablets equivalent to 0.5 g of Quinalbarbitone Sodium with 10 ml of *water*, filter, acidify the filtrate with *acetic acid*; oily drops are formed which may eventually crystallise.

(C) The powdered tablets give the reactions of *sodium*, Appendix 3.1.

Disintegration : Maximum time, 15 minutes, Appendix 5.6.1.

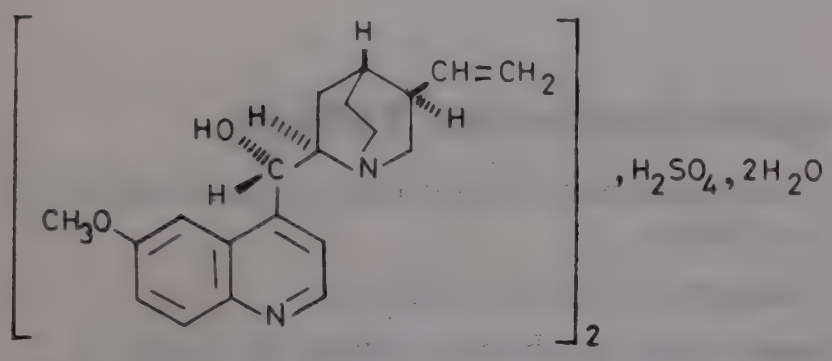
Other requirements : Comply with the requirements stated under Tablets.

Assay : Digest 20 tablets with 50 ml of *water* until completely disintegrated and not more than a small residue remains. Add 5 ml of *N sodium hydroxide*, filter, and wash the residue with sufficient *water* to produce 100.0 ml. Extract a volume of the solution equivalent to 0.5 g of Quinalbarbitone Sodium with two quantities, each of 15 ml, of *solvent ether*, washing each ether extract with the same 3 ml of *water*. Add the *water* to the aqueous liquid and acidify with *hydrochloric acid*, and extract with successive

quantities, each of 15 ml, of *solvent ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 2 ml, of *water* and extract the combined washings with 10 ml of *solvent ether*. Add the ether to the main ether layer, filter, and wash the filter with *solvent ether*. Evaporate the solvent and dry the residue to constant weight at 50°. Each g of residue is equivalent to 1.092 g of $C_{12}H_{17}N_2NaO_3$.

Storage : Store in tightly-closed containers, protected from moisture.

Quinidine Sulphate



$(C_{20}H_{24}N_2O_2)_2, H_2SO_4, 2H_2O$ Mol. Wt. 782.95

Category : Cardiac depressant (anti-arrhythmic).

Dose : In the prophylaxis of cardiac arrhythmias, 0.2 g daily, three or four times daily. In the treatment of atrial fibrillation, 0.2 to 0.4 g every two to four hours to a total dose of 3 g daily.

Description : White, needle-like crystals; odourless; taste, very bitter.

Solubility : Sparingly soluble in *water*; soluble in *alcohol* and in *chloroform*; practically insoluble in *solvent ether*.

Standards : Quinidine Sulphate is the dihydrate of (8*R*, 9*S*)-6′-methoxycinchonan-9-ol-sulphate, obtained from the bark of various species of *Cinchona* and from *Remijia pedunculate* Fluckiger (Fam. Rubiaceae) or prepared from quinine. It contains not less than 99.0 per cent and not more than the equivalent of 101.5 per cent of $(C_{20}H_{24}N_2O_2)_2, H_2SO_4$, calculated with reference to the dried substance.

Identification : (A) To 5 ml of a 0.1 per cent w/v solution add 2 or 3 drops of *bromine solution* and then 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

(B) To a 0.5 per cent w/v solution, add an equal volume of *dilute sulphuric acid*; a strong blue fluorescence is produced.

(C) To 5 ml of a 1.0 per cent w/v solution, add 1 ml of *silver nitrate solution*, and stir with a glass rod; after a short interval, a white precipitate, soluble in *nitric acid* is produced (distinction from many other alkaloids).

(D) A solution (1 in 100) gives the reactions of *sulphates*, Appendix 3.1.

Specific optical rotation : Between +275° and +287°, determined in a 2.0 per cent w/v solution in *0.1N hydrochloric acid*, Appendix 5.12.

pH : Between 6.0 and 6.8, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Dihydroquinidine sulphate : Not more than 15.0 per cent, calculated with reference to the dried substance and determined by the following method: Dissolve 0.2 g in 20 ml of *water* and add 0.5 g of *potassium bromide* and 15 ml of *2N hydrochloric acid*. Titrate slowly with *0.1N potassium bromate* using *methyl red solution* as indicator, until a yellow colour is obtained. Add a solution of 0.5 g of *potassium iodide* in 200 ml of *water* and stopper the flask immediately. Allow to stand in the dark for five minutes and titrate with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. Each ml of *0.1N potassium bromate* is equivalent to 0.01867 g of $(C_{20}H_{24}N_2O_2)_2, H_2SO_4$. Calculate the content of dihydroquinidine sulphate by subtracting the result from the assay result.

Other cinchona alkaloids : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 20 volumes of *toluene*, 12 volumes of *ether* and 5 volumes of *diethylamine* as the mobile phase. Apply separately to the plate 4 µl of each of four solutions in *methyl alcohol* containing (1) 1.0 per cent w/v of the substance being examined; (2) 0.025 per cent w/v of *quinine*; (3) 0.025 per cent w/v of *cinchonine*; and (4) 1.0 per cent w/v of *quinidine sulphate* and 0.025 per cent w/v of *cinchonine*. After removal of the plate, allow it to dry in air for fifteen minutes and repeat the development. Dry the plate at 105° for thirty minutes, allow to cool and spray with *potassium iodoplatinate solution*. Any spot in the chromatogram obtained with solution (1), other than the principal spot, is not more intense than the spots in the chromatograms obtained with solutions (2) and (3). The test is valid only if the chromatogram obtained with solution (4) shows two clearly separated spots.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Between 3.0 per cent and 5.0 per cent, determined on 1.0 g by drying in an oven at 130°, Appendix 5.8.

Assay : Weigh accurately about 0.4 g and dissolve in 40 ml of *acetic anhydride*. Add a few drops of *crystal-violet solution* and titrate with *0.1N perchloric acid* to a blue-green end-point. Carry out a blank determination

and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02490 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

Storage : Store in well-closed, light-resistant containers.

Quinidine Tablets

Quinidine Sulphate Tablets

Category : Cardiac depressant (anti-arrhythmic).

Dose : Quinidine Sulphate. In the prophylaxis of cardiac arrhythmias, 0.2 g daily, three or four times daily. In the treatment of auricular fibrillation, 0.2 to 0.4 g every two to four hours to a total dose of 3 g daily.

Usual strength : 0.2 g.

Standards : Quinidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Quinidine Sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Identification : Extract a quantity of the powdered tablets equivalent to 0.1 g of Quinidine Sulphate with 20 ml of *water* and filter. The filtrate complies with the following tests:

(i) It is *dextro*-rotatory.

(ii) To 1 ml add 4 ml of *water*, 2 or 3 drops of *bromine solution* and then 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

(iii) It gives the reactions of *sulphates*, Appendix 3.1.

Dissolution : Carry out the *dissolution test for tablets and capsules*, Appendix 5.7, using as the medium 1000 ml of *0.1N hydrochloric acid*, placing one tablet in the basket for each test and rotating the basket for thirty minutes. Withdraw a sample of the medium and filter. Measure the *extinction* of the filtered solution, suitably diluted if necessary, at the maximum at about 248 nm, Appendix 5.15 A. Calculate the content of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$, in the medium from the *extinction* obtained from a solution of known concentration of *quinidine sulphate R.S.* and from the declared content of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$ in the *quinidine sulphate R.S.*

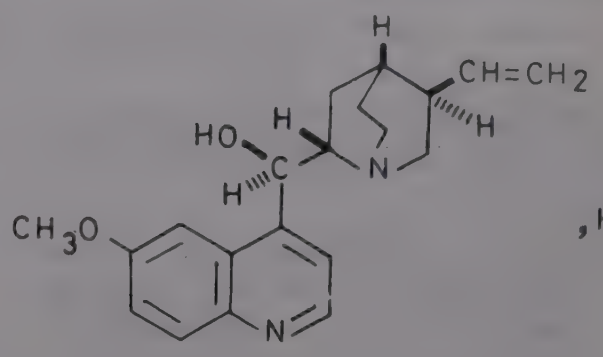
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.4 g of Quinidine Sulphate, dissolve as completely as possible in 40 ml of *acetic anhydride* with the aid of heat and cool. Complete the **Assay** as described under Quinidine Sulphate, beginning at the words "add a few drops of *crystal-violet solution*". Each ml of *0.1N perchloric acid* is equivalent to 0.02610 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Storage : Store in well-closed, light-resistant containers.

Quinine Bisulphate

Quinine Acid Sulphate



$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$ Mol. Wt. 548.60

Category : Antimalarial.

Dose : In the suppression of malaria, 0.3 to 0.6 g daily. In the treatment of malaria, 1.2 to 2 g daily, in divided doses.

Description : Colourless or faintly yellow crystals or white or faintly yellow, crystalline powder; odourless; taste, very bitter. Efflorescent in dry air.

Solubility : Soluble in *water*; sparingly soluble in *alcohol*; slightly soluble in *chloroform*. Freely soluble in boiling *water* and in boiling *alcohol*.

Standards : Quinine Bisulphate is the heptahydrate of (8*S*,9*R*)-6'-methoxycinchonan-9-ol hydrogen sulphate. It contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of total alkaloids, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$, with reference to the dried substance.

Identification : (A) To 5 ml of a 0.1 per cent w/v solution, add two or three drops of *bromine solution* and then 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

(B) A 5.0 per cent w/v solution has a blue fluorescence.

(C) A solution (1 in 20) gives the reactions of *sulphates*, Appendix 3.1.

pH : Between 2.8 and 3.4, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Specific optical rotation : Between −208° and −216°, determined at 20° in a 3 per cent w/v solution in *0.1N hydrochloric acid*, Appendix 5.12.

Other cinchona alkaloids : Complies with the test described under Quinidine Sulphate, using a 0.025 per cent w/v solution of *cinchonidine* as solution (2).

Readily carbonisable substances : Dissolve 0.25 g in 5 ml of *sulphuric acid* (having 94.5 to 95.5 per cent of H_2SO_4); the solution is not more intensely coloured than a solution prepared by mixing 0.1 ml of *cobalt chloride C.S.*, 2.0 ml of *ferric chloride C.S.*, 0.1 ml of *copper sulphate C.S.* and 2.8 ml of *water*.

Ammonium salts : Heat 0.3 g with 3 ml of *sodium hydroxide solution* on a water-bath; the mixture does not at once evolve the odour of ammonia.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Between 19.0 per cent and 24.0 per cent, determined on 0.5 g by drying "in vacuo at 60°" for eighteen hours, Appendix 5.8.

Assay : Dissolve 0.45 g in 15 ml of *water*. Add 25 ml of *0.1N sodium hydroxide* and extract with three quantities, each of 25 ml, of *chloroform*. Wash the combined chloroform extracts with 20 ml of *water*. Dry the chloroform extracts with *anhydrous sodium sulphate*, evaporate to dryness under reduced pressure and dissolve the residue in 50 ml of *glacial acetic acid*. Add a few drops of *crystal violet solution* and titrate with *0.1N perchloric acid*. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02113 g of $C_{20}H_{24}N_2O_2, H_2SO_4$.

Storage : Store in well-closed, light-resistant containers.

Quinine Bisulphate Tablets

Quinine Acid Sulphate Tablets

Category : Antimalarial.

Dose : Quinine Bisulphate. In the suppression of malaria, 0.3 to 0.6 g daily. In the treatment of malaria, 1.2 to 2 g daily, in divided doses.

Usual strength : 0.3 g.

Standards : Quinine Bisulphate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Quinine Bisulphate, $C_{20}H_{24}N_2O_2, H_2SO_4, 7H_2O$. The tablets may be coated.

Identification : Shake a quantity of the powdered tablets equivalent to 0.5 g of Quinine Bisulphate with 10 ml of *water* and filter; the filtrate has a blue fluorescence and complies with the following tests:

(i) It is *laevo*-rotatory and gives a strongly acid reaction to *bromocresol purple solution*, but not to *congo red solution*.

(ii) Dilute 0.1 ml to 5 ml with *water* and add 2 or 3 drops of *bromine solution* and then 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

(iii) It gives the reactions of *sulphates*, Appendix 3.1.

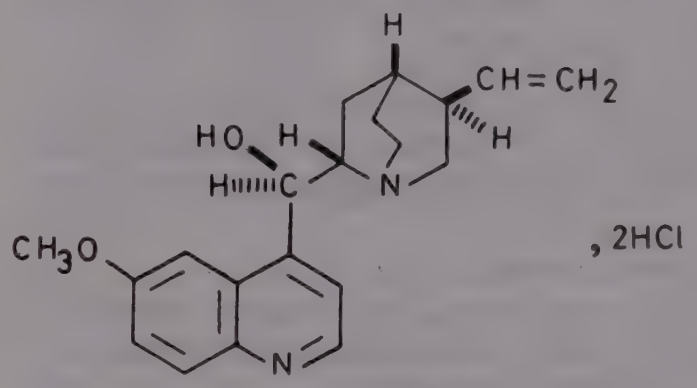
Other requirements : Comply with the requirements stated under Tablets.

Assay : Carry out the **Assay** described under Quinidine Sulphate Tablets, using an accurately weighed quantity of the powdered tablets equivalent to about 0.6 g of Quinine Bisulphate. Each ml of *0.1N perchloric acid* is equivalent to 0.05486 g of $C_{20}H_{24}N_2O_2, H_2SO_4, 7H_2O$.

Storage : Store in well-closed, light-resistant containers.

Quinine Dihydrochloride

Quinine Acid Hydrochloride



$C_{20}H_{24}N_2O_2, 2HCl$ Mol. Wt. 397.34

Category : Antimalarial.

Dose : In the suppression of malaria, 0.3 to 0.6 g daily. In the treatment of malaria, 1.2 to 2 g daily, in divided doses. By slow intravenous injection, 0.3 to 0.6 g.

Description : White or nearly white powder; odourless; taste, very bitter.

Solubility : Very soluble in *water*; soluble in *alcohol*; slightly soluble in *chloroform* and very slightly soluble in *solvent ether*.

Standards : Quinine Dihydrochloride is the dihydrochloride of (8*S*, 9*R*)-6′-methoxycinchonan-9-ol obtained from the bark of various species of Cinchona. It contains not less than 99.0 per cent and not more than the equivalent of 101.5 per cent of $C_{20}H_{24}N_2O_2, 2HCl$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** tests (A) and (B) described under Quinidine Sulphate.

(B) lution (1 in 20) gives the reactions of *chlorides*, idix 3.1.

Specific optical rotation : Between −224° and −230°, determined in a 3.0 per cent w/v solution in *0.1N hydrochloric acid*, Appendix 5.12.

pH : Between 2.0 and 3.0, determined in a 3.0 per cent w/v solution, Appendix 5.10.

Barium : To 15 ml of a 2 per cent w/v solution, add 1 ml of *dilute sulphuric acid*; the solution remains clear for at least fifteen minutes.

Sulphate : 1.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Other cinchona alkaloids : Complies with the test described under Quinidine Sulphate, using 0.25 per cent w/v solution of *cinchonidine* as solution (2).

Readily carbonisable substances : Complies with the test described under Quinine Bisulphate.

Dihydroquinine dihydrochloride : Not more than 10.0 per cent, calculated with reference to the dried substance and determined by the method described under Quinidine Sulphate. Each ml of *0.1N potassium bromate* is equivalent to 0.01987 g of $C_{20}H_{24}N_2O_2, 2HCl$. Calculate the content of dihydroquinine dihydrochloride by subtracting the result from the assay result.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.3 g, dissolve in 50 ml of *glacial acetic acid*, add 20 ml of *acetic anhydride* and 10 ml of *mercuric acetate solution* and complete the **Assay** described under Quinidine Sulphate, beginning at the words "add a few drops of *crystal-violet solution*.....". Each ml of *0.1N perchloric acid* is equivalent to 0.01987 g of $C_{20}H_{24}N_2O_2, 2HCl$.

Storage : Store in well-closed, light-resistant containers.

Quinine Dihydrochloride Injection

Category : Antimalarial.

Dose : Quinine Dihydrochloride. By slow intravenous injection, 0.3 to 0.6 g.

Usual strength : 0.3 g per ml.

Description : Clear, almost colourless to light yellow solution.

Standards : Quinine Dihydrochloride Injection is a sterile solution of Quinine Dihydrochloride in Water for Injection. It contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_{20}H_{24}N_2O_2, 2HCl$.

Identification : (A) Complies with **Identification** tests (A) and (B) described under Quinidine Sulphate.

(B) It gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 1.5 and 3.0, Appendix 5.10.

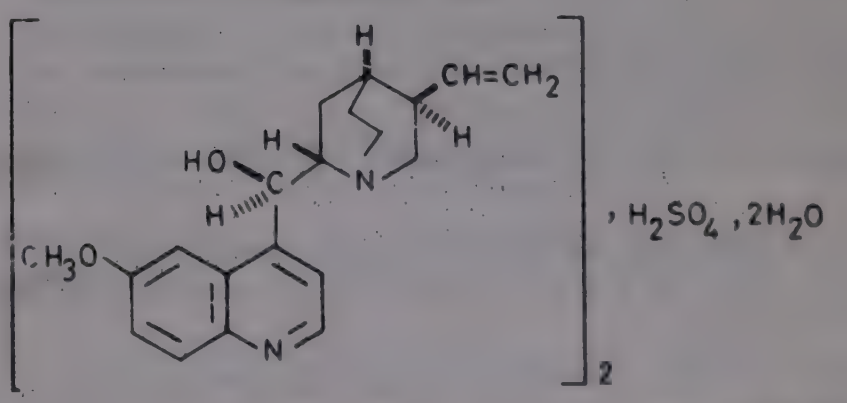
Other requirements : Complies with the requirements stated under Injections.

Assay : To an accurately measured volume equivalent to about 0.5 g of Quinine Dihydrochloride, add 20 ml of *water*, and 5 ml of *sodium hydroxide solution*, and extract with successive quantities, each of 10 ml, of *chloroform* until complete extraction of the alkaloid is effected, washing each extract with the same two quantities, each of 5 ml, of *water*. Remove the chloroform from the combined extracts, dissolve the residue in 50 ml of *glacial acetic acid*, add 20 ml of *acetic anhydride* and 10 ml of *mercuric acetate solution* and complete the **Assay** as described under Quinidine Sulphate, beginning at the words "add a few drops of *crystal-violet solution*.....". Each ml of *0.1N perchloric acid* is equivalent to 0.01987 g of $C_{20}H_{24}N_2O_2, 2HCl$.

Storage : Store in single-dose or multiple-dose, light-resistant containers.

Labelling : The label on the container states that the solution must be diluted to a strength, not exceeding 0.03 g per ml before administration and that care should be taken to ensure slow intravenous injection.

Quinine Sulphate



$(C_{20}H_{24}N_2O_2)_2, H_2SO_4, 2H_2O$ Mol. Wt. 782.95

Category : Antimalarial.

Dose : In the suppression of malaria, 0.3 to 0.6 g daily. In the treatment of malaria, 1.2 to 2 g daily, in divided doses.

Description : White, needle-like crystals; odourless; taste, very bitter.

Solubility : Slightly soluble in *water*, in *alcohol*, in *chloroform* and in *solvent ether*; freely soluble in a mixture of two volumes of *chloroform* and one volume of *ethyl alcohol*.

Standards : Quinine Sulphate is the dihydrate of (8*S*, 9*R*)-6′-methoxycinchonan-9-ol sulphate obtained from the bark of various species of *Cinchona*. It contains not less than 99.0 per cent and not more than the equivalent of 101.5 per cent of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** tests (A) and (D) described under Quinidine Sulphate.

(B) To a few drops of a saturated solution add one drop of *dilute sulphuric acid*; a strong blue fluorescence is produced.

Specific optical rotation : Between −240° and −248°, determined in a 2.0 per cent w/v solution in *0.1N hydrochloric acid*, Appendix 5.12.

pH : Between 5.7 and 6.6, determined in a 1.0 per cent w/v suspension in *water*, Appendix 5.10.

Inorganic salts and other alkaloids : 1.0 g dissolves completely in 7 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethyl alcohol* when heated to 50°; the solution remains clear on standing.

Other cinchona alkaloids : Complies with the test described under Quinidine Sulphate, using a 0.025 per cent w/v solution of *cinchonidine* as solution (2).

Readily carbonisable substances : Complies with the test described under Quinine Bisulphate.

Dihydroquinine sulphate : Not more than 10.0 per cent, calculated with reference to the dried substance and determined by the method described under Quinidine Sulphate. Each ml of *0.1N potassium bromate* is equivalent to 0.01867 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$. Calculate the content of dihydroquinine sulphate by subtracting the result from the assay result.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Between 3.0 per cent and 5.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the **Assay** described under Quinidine Sulphate.

Storage : Store in well-closed, light-resistant containers.

Quinine Sulphate Tablets

Category : Antimalarial.

Dose : Quinine Sulphate. In the suppression of malaria, 0.3 to 0.6 g daily.

In the treatment of malaria, 1.2 to 2 g daily, in divided doses.

Usual strength : 0.3 g.

Standards : Quinine Sulphate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Quinine Sulphate $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$. The tablets are coated.

Identification : Extract a quantity of the powdered tablets equivalent to 0.1 g of Quinine Sulphate with 20 ml of *water* and filter; the filtrate complies with the following tests:

(i) It is *laevo*-rotatory and is not acid to *bromocresol purple solution*.

(ii) To 5 ml add 2 or 3 drops of *bromine solution* and then 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

(iii) It gives the reactions of *sulphates*, Appendix 3.1.

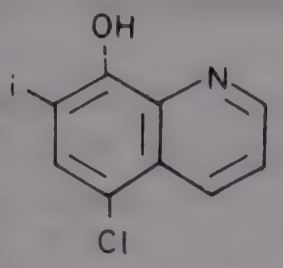
Other requirements : Comply with the requirements stated under Tablets.

Assay : Carry out the **Assay** described under Quinidine Tablets.

Storage : Store in well-closed, light-resistant containers.

Quiniodochlor

Iodochlorhydroxyquinoline; Clioquinol



C_9H_5ClINO Mol. Wt. 305.50

Category : Antiprotozoal; topical and intestinal antiseptic.

Dose : 0.75 to 1.5 g daily, in divided doses.

Description : Yellowish-white to brownish-yellow powder; odour, faint and characteristic; tasteless.

Solubility : Practically insoluble in *water*; sparingly soluble in *alcohol*; soluble in hot *glacial acetic acid*, in hot *ethyl acetate*, in *dimethylformamide* and in *pyridine*.

Standards : Quiniodochlor is 5-chloro-7-iodoquinolin-8-ol. It contains not less than 93.0 per cent and not more than the equivalent of 101.0 per cent of C_9H_5ClINO, calculated with reference to the dried substance.

Identification : (A) Heat 0.1 g with 5.0 ml of *sulphuric acid*; copious violet vapours of iodine are evolved.

(B) To 5 ml of a 1.0 per cent w/v suspension in *water* add 5 ml of *dilute hydrochloric acid*, 5 ml of *chloroform* and 1 drop of a 10 per cent w/v solution of *sodium nitrite* and shake; the chloroform is coloured violet.

(C) *Extinction* of a 1-cm layer of a 0.001 per cent w/v solution in *3N hydrochloric acid* at the maximum at about 267 nm, about 0.88, Appendix 5.15A.

Melting range : Between 172° and 182°, with decomposition, Appendix 5.11.

Acidity or Alkalinity : Shake 0.5 g with 10 ml of *water* previously neutralised to *phenolphthalein solution*; the solution is colourless, but turns pink on adding not more than 0.05 ml of *0.1N sodium hydroxide*.

Free iodine and iodide : Shake 1.0 g with a solution of 1 g of *potassium iodide* in 20 ml of *water* for thirty seconds, allow to stand for five minutes and filter. To 10 ml of the filtrate add 1 ml of *dilute sulphuric acid* and 2 ml of *chloroform* and shake; no violet colour appears in the chloroform layer (free iodine). To the mixture add 5 ml of *2N sulphuric acid* and 1 ml of a 7.5 per cent w/v solution of *potassium dichromate* and shake for fifteen seconds; the colour in the chloroform layer is not more intense than that produced in a solution prepared by diluting 2.0 ml of a solution of 0.01 g of *potassium iodide* in 60 ml of *water* to 10 ml and adding 6 ml of *2N sulphuric acid*, 1 ml of a 7.5 per cent w/v solution of *potassium dichromate* and 2 ml of *chloroform* and shaking for fifteen minutes.

Halide ions : Shake 0.50 g with 25 ml of *water* for one minute and filter; to the filtrate add 0.5 ml of *dilute nitric acid* and 0.5 ml of *0.1N silver nitrate* and allow to stand for five minutes. The opalescence produced is not greater than that produced five minutes after the addition of 0.5 ml of *0.1N silver nitrate* to 25 ml of *water* containing 0.5 ml of *dilute nitric acid* and 0.2 ml of *0.01N hydrochloric acid*.

Chlorine and iodine content : Between 11.0 per cent and 12.2 per cent of chlorine (Cl) and between 39.0 and 42.0 per cent of iodine (I), both calculated with reference to the dried substance and determined by the following method: Carry out the *oxygen-flask method*, Appendix 3.3.6, using about 20 mg, accurately weighed, and a mixture of 5 ml of a 1 per cent w/v solution of *sodium hydroxide* and 3 ml of a saturated solution of *sulphur dioxide* as the absorbing liquid. When the process is complete, transfer the contents of the flask to a beaker with the aid of four quantities, each of 5 ml, of a buffer solution prepared by dissolving 13.61 g of *sodium acetate* in 50 ml of *water* and adding 6.0 ml of *glacial acetic acid* and sufficient *water* to produce 100 ml. Add 25 ml of *acetone* and one drop of a suitable non-ionic wetting agent such as *p*-nonylphenyl polyethyleneglycol and titrate the iodine and chlorine in the solution with *0.1N silver nitrate*, determining the end-point potentiometrically, using a silver electrode and a mercuric sulphate-potassium sulphate reference electrode. Add *0.1N silver nitrate* in aliquote of 10µl, stir vigorously and after each addition plot the observed electromotive force against volume of titrant. Continue the titration through two points of inflection, one of which should occur between 430 and 410 millivolts and the other between 185 and 160 millivolts. Each ml of *0.1N silver nitrate* required in reaching the first inflection point is equivalent to 0.01269 g of I and each ml required between the first inflection point and the second is equivalent to 0.003545 g of Cl.

Residue on ignition : Not more than 0.5 per cent, determined, by igniting to constant weight at red heat.

Loss on drying : Not more than 0.5 per cent determined on 1.0 g by drying "in vacuo" for five hours, Appendix 5.8.

Assay : Weigh accurately about 0.15 g, previously dried, dissolve in *carbon disulphide* and dilute to 10.0 ml with *carbon disulphide*. Dissolve an accurately weighed quantity of *quiniodochlor R.S.* in *carbon disulphide* and dilute quantitatively with *carbon disulphide* to obtain a solution having a concentration of about 15 mg per ml. Determine the average *extinction* of the two solutions in 1 mm cells at the wavelength of maximum absorption at about 14.4 µm (694 cm^{-1}) and of minimum absorption at about 14.9 µm (671 cm^{-1}) with a suitable infra-red spectrophotometer, using *carbon disulphide* as the blank, Appendix 5.15B. Calculate the quantity in mg of C_9H_5ClINO in the sample from the formula $10\,C(Eu_{14.4}-Eu_{14.9})/(Es_{14.4}-Es_{14.9})$, in which C is the concentration, in mg per ml of *quiniodochlor R.S.* in the standard preparation, and Eu and Es are the average *extinctions* of the sample and the standard preparations respectively, at the wavelengths indicated by the subscripts.

Storage : Store in well-closed, light-resistant containers.

Quiniodochlor Tablets

Iodochlorhydroxyquinoline Tablets

Category : Anti-amoebic.

Dose : Quiniodochlor; 250 to 750 mg daily, in divided doses.

Usual strength : 250 mg.

Standards : Quiniodochlor Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Quiniodochlor, C_9H_5ClINO.

Identification : Triturate a quantity of the powdered tablets equivalent to about 250 mg of Quiniodochlor with 20 ml of *acetone* and filter. Add 20 ml of *water* to the filtrate. Collect the precipitate on a filter and dry at 100°. The precipitate melts at about 178°, Appendix 5.11 and complies with **Identification** tests (A) and (B) described under Quiniodochlor.

Disintegration : Maximum time, thirty minutes, Appendix 5.6.1.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 100 mg of Quiniodochlor, add 50 ml of *ethyl acetate* and heat to boiling. Decant the hot supernatant liquid through a filter. Repeat the extraction with three further quantities, each of 50 ml of *ethyl acetate*, washing the residue and the filter. Combine the extracts and evaporate on a water-bath to dryness. Dissolve the residue in about 40 ml of *acetone*, add 2 ml of *glacial acetic acid* and 20 ml of a saturated solution of *copper acetate* in *methyl alcohol*. Allow to stand for five minutes, and filter through a sintered glass crucible (grade 4, maximum pore size, 5 to 15μ) and wash the residue with five quantities, each of 5 ml, of *acetone* followed by 5 quantities, each of 5 ml, of *hexane*. Dry the residue to constant weight at 105°. Each g of residue is equivalent to 0.91 g of C_9H_5ClINO.

Storage : Store in light-resistant containers.

Rabies Antiserum

Antirabies Serum; Antirabic Serum

Category : Immunising agent.

Dose : By intramuscular or subcutaneous injection, 40 International Units per kg of body weight.

Description : Almost colourless or faintly yellow, slightly opalescent liquid, free from suspended particles.

Standards : Rabies Antiserum is a preparation containing the specific antirabies globuline obtained by purification from native serum and having the specific power of neutralising the virus of rabies.

It contains the specific neutralising power equivalent to not less than 80 International Units per ml.

The vaccine may contain a suitable antimicrobial agent.

Identification : It specifically neutralises the rabies virus, rendering it harmless to susceptible animals.

Other requirements : Complies with the requirements for general tests stated under Antisera.

Potency : Carry out the *biological assay of rabies antiserum*, Appendix 2.18.

Storage : Store in single-dose or multiple-dose containers at a temperature between 2° and 8°, protected from light. Do not allow it to freeze.

Labelling : The label on the container states (1) the number of Units per ml; (2) the dose; (3) the total volume in the container; (4) the name and proportion of any added antibacterial substances; (5) the species of animal used in preparing the antiserum; (6) the storage conditions; (7) the date after which the preparation is not intended to be used.

Rabies Vaccine

Category : Specific immunising agent.

Dose : By subcutaneous injection. 1 to 5 ml daily, for seven to fourteen days according to the site and severity of the bite and the risk of exposure to infection.

Description : White, flocculent suspension in a clear liquid, or white to brownish-white turbid liquid.

Standards : Rabies Vaccine is a sterile suspension in saline or other appropriate solution, isotonic with blood, of a suitable killed rabies virus in uncontaminated brain tissue from animals previously injected intracerebrally with rabies virus.

It may be prepared by injecting sheep, rabbits, suckling rats, mice or other suitable animals intracerebrally with rabies virus. Only these animals which show typical paralysis are killed, the brains are harvested under aseptic condition, tested for absence of bacteria and then finally suspended by homogenisation in Sodium Chloride Injection or any other suitable liquid. The suspension is inactivated by the addition of Phenol or Betapropiolactone or by any

other means until the virus will no longer infect mice. It is diluted to contain not more than 5 per cent w/v of brain material and not more than 0.25 per cent w/v of phenol or not more than 0.04 per cent w/v of betapropiolactone. The finished vaccine may contain a suitable antibacterial substance such as thiomersal in a concentration not exceeding 0.01 per cent w/v and sufficient neutralisers to adjust the pH between 7.0 and 7.2.

The final product is distributed under aseptic condition into previously sterilised containers, which are sealed so as to exclude micro-organisms.

Identification : It protects susceptible animals against subsequent infection by an authentic strain of rabies virus.

Absence of line virus : Inject 0.03 ml intracerebrally into each of not fewer than ten mice weighing between 14 and 16 g neither symptoms of disease in the central nervous system nor death occurs in any of the animals within fourteen days.

pH : Between 7.0 and 7.4, Appendix 5.10.

Phenol (if present) : Not more than 0.25 per cent w/v, Appendix 3.3.9.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

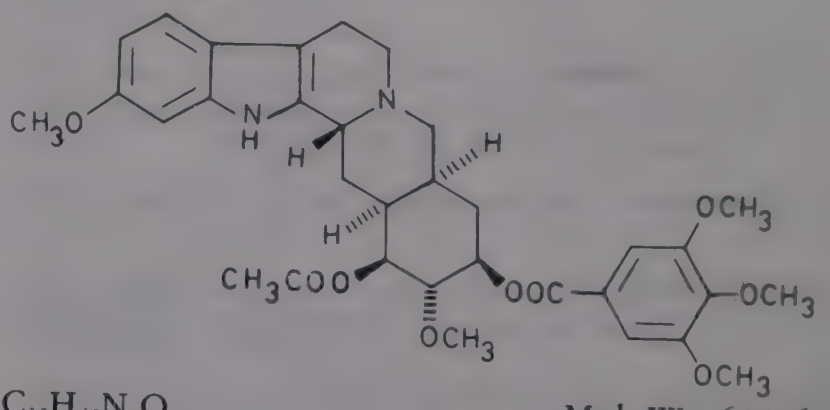
Undue toxicity : Complies with the *test for undue toxicity of vaccines and sera*, Appendix 2.37, using 0.5 ml for the mice and 5.0 ml for the guinea-pigs.

Assay : Carry out the *biological assay of rabies vaccine*, Appendix 2.24.

Storage : Store in containers, protected from light and at a temperature between 2° and 8° It should not be used until the absence of live virus has been established.

Labelling : The label on the container states (1) the recommended dose and number of doses in the container; (2) the nature and concentration of any added antibacterial substance; (3) the storage conditions; (4) the date after which it is not intended to be used.

Reserpine



$C_{33}H_{40}N_2O_9$ Mol. Wt. 608.69

Category : Major tranquilliser; antihypertensive.

Dose : In psychiatric states, 1 to 5 mg daily, in divided doses. In the treatment of hypertension, 0.5 mg daily.

Description : White or pale buff to slightly yellowish, crystalline powder; odourless; almost tasteless.

Solubility : Practically insoluble in *water*, and in *solvent ether;* very slightly soluble in *alcohol*; sparingly soluble in *acetone*; freely soluble in *chloroform*; slightly soluble in *methyl alcohol.*

Standards : Reserpine is methyl 11,17α- dimethoxy-18β-(3,4,5-trimethoxybenzoyloxy)-3β,20α-yohimbane-16β-carboxylate, an alkaloid obtained from certain species of *Rauvolfia*, mainly *Rauvolfia serpentina* Benth. (Fam. Apocyanaceae) or by synthesis. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of the total alkaloids, and not less than 97.0 per cent and not more than the equivalent of 102.0 per cent of reserpine, $C_{33}H_{40}N_2O_9$, both calculated with reference to the dried substance.

Identification : (A) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.002 per cent w/v solution in *alcohol* exhibits a maximum at 268 nm; *extinction* at 268 nm, about 0.55 over the range 288 to 925 nm, the *extinction* of the solution is about 0.34, Appendix 5.15 A.

(B) To 1 mg add 0.1 ml of a 0.1 per cent w/v solution of *sodium molybdate* in *sulphuric acid*; a yellow colour is produced immediately, and in about two minutes it changes to blue.

(C) To 1 mg add 0.2 ml of a freshly prepared 1 per cent w/v solution of *vanillin* in *hydrochloric acid*; a rose-pink colour is produced in about two minutes.

(D) Mix 0.5 mg with 5 mg of *dimethylaminobenzaldehyde* and 0.2 ml of *glacial acetic acid* and add 0.2 ml of *sulphuric acid*; the colour changes to red.

(E) It melts at about 270°, with decomposition, Appendix 5.11, the tube being placed in the heating bath at 250°.

Specific optical rotation : Between −113° and −123°, determined in a 1 per cent w/v solution in *chloroform*, Appendix 5.12.

Oxidation products : *Extinction* of a 1-cm layer of a 0.02 per cent w/v solution in *glacial acetic acid* at 388 nm; not greater than 0.10, Appendix 5.15 A.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying "in vacuo at 60°" for 3 hours, Appendix 5.8.

Assay : *For total alkaloids*—Weigh accurately about 0.15 g, dissolve in 30 ml of warm *glacial acetic acid*, cool, and titrate with *0.1N perchloric acid*, using *quinaldine red solution* as indicator. Perform a blank titration and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.06087 g of total alkaloids.

For $C_{33}H_{40}N_2O_9$—Protect the solutions from light throughout the Assay.

Weigh accurately about 20 mg, moisten with 2 ml of *alcohol*, add 2 ml of *0.5N sulphuric acid* and 10 ml of *alcohol*, and warm gently to effect solution. Cool, dilute to 100.0 ml with *alcohol* and dilute 5.0 ml of this solution to 50.0 ml with *alcohol*. Transfer 10.0 ml of the solution to a boiling tube, add 2 ml of *0.5N sulphuric acid* and 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix, and heat in a water-bath at 55° for thirty minutes. Cool, add 1.0 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid*, and dilute to 20.0 ml with *alcohol*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 390 nm, Appendix 5.15 A, using as the blank a solution prepared by treating a further 10 ml of the solution in the same manner but omitting the sodium nitrite solution. Calculate the content of $C_{33}H_{40}N_2O_9$ from the *extinction* obtained by repeating the operation using *reserpine R.S.* instead of the substance being examined, and from the declared content of $C_{33}H_{40}N_2O_9$ in the *reserpine R.S.*

Storage : Store in well-closed, light-resistant containers.

Reserpine Injection

Category : Major tranquilliser; antihypertensive.

Dose : Reserpine. By intramuscular injection, 0.25 to 1 mg.

Usual strengths : 1.0 mg per ml; 2.5 mg per ml.

Description : Clear, colourless liquid.

Standards : Reserpine Injection is a sterile solution of Reserpine in Water for Injection, prepared with the aid of a suitable acid. It contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Reserpine, $C_{33}H_{40}N_2O_9$. It contains suitable antioxidants.

Identification : Extract a volume equivalent to 2.5 mg of Reserpine with 10 ml of *chloroform* and evaporate the chloroform layer to dryness. The residue complies with the **Identification** tests (A) to (D) described under Reserpine.

pH : Between 3.0 and 4.0, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

Assay : Protect the solutions from light throughout the Assay.

Measure accurately a volume equivalent to 10 mg of Reserpine and dilute with a 2 per cent w/v solution of *citric acid* to 100.0 ml. Extract 10.0 ml of this solution for two minutes with three quantities, each of 15 ml, of *chloroform*. Wash the combined extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate*, add sufficient *chloroform* to produce 50.0 ml, and evaporate 10.0 ml to dryness on a water-bath. Dissolve the residue in 10 ml of *alcohol* and 2 ml of *0.5N sulphuric acid* with the aid of gentle heat, add 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix, and heat in a water-bath at 55° for thirty minutes. Cool, add 1.0 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid*, and dilute to 20.0 ml with *alcohol*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 390 nm, Appendix 5.15 A, using as the blank, a solution prepared by treating 10 ml of the chloroform solution in the same manner but omitting the sodium nitrite solution. Calculate the content of $C_{33}H_{40}N_2O_9$, from the *extinction* obtained by repeating the operation using *reserpine R.S.* instead of the substance being examined and from the declared content of $C_{33}H_{40}N_2O_9$ in the *reserpine R.S.*

Storage : Store in single-dose (or if stabilisers are present, in multiple-dose), light-resistant containers.

Reserpine Tablets

Category : Major tranquilliser; antihypertensive.

Dose : Reserpine. In psychiatric states, 1 to 5 mg daily, in divided doses. In the treatment of hypertension, 0.5 mg daily.

Usual strengths : 0.1 mg; 0.25 mg; 0.5 mg; 1 mg.

Standards : Reserpine Tablets contain not less than

90.0 per cent and not more than 110.0 per cent of the stated amount of Reserpine, $C_{33}H_{40}N_2O_9$.

Identification : Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness; the residue complies with the **Identification** tests (B), (C) and (D) described under Reserpine.

Uniformity of content : Protect the solutions from light throughout the test.

Powder one tablet, disperse in 10 ml of a 2% w/v solution of *citric acid* and extract with three quantities, each of 5 ml, of *chloroform*; filter the extracts through a plug of cotton wool moistened with *chloroform*. Wash the chloroform extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate*, and evaporate the chloroform extracts to dryness on a water-bath. Carry out the **Assay** as described under Reserpine Injection beginning at the words "Dissolve the residue. . . .". Calculate the content of $C_{33}H_{40}N_2O_9$ in the tablet.

Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 and 110 per cent of the average, except that for one tablet the content may be between 85 and 115 per cent of the average.

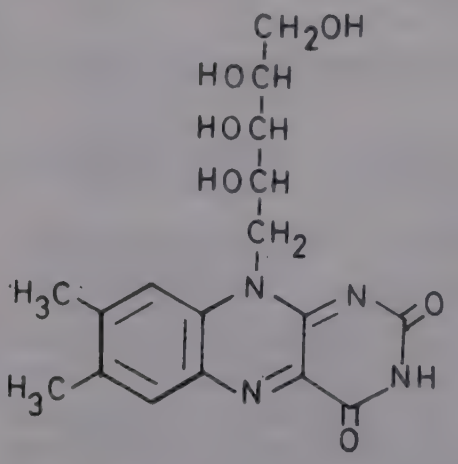
Other requirements : Comply with the requirements stated under Tablets.

Assay : Protect the solutions from light throughout the assay. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 1 mg of Reserpine, add 10 ml of a 2 per cent w/v solution of *citric acid* and extract for two minutes with three quantities, each of 15 ml, of *chloroform*. Wash the combined extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate*, add sufficient *chloroform* to produce 50.0 ml and evaporate 10.0 ml to dryness on a water-bath. Carry out the **Assay** as described under Reserpine Injection, beginning at the words "Dissolve the residue. . . . . ". Calculate the content of $C_{33}H_{40}N_2O_9$ in the tablets from the *extinction* obtained by repeating the operation using *reserpine R.S.* instead of the powder and from the declared content of $C_{33}H_{40}N_2O_9$ in the *reserpine R.S.*

Storage : Store in well-closed, light-resistant containers.

Riboflavine

Vitamin B_2; Lactoflavin



$C_{17}H_{20}N_4O_6$ Mol. Wt. 376.37

Category : B-group Vitamin.

Dose : Prophylactic, 1 to 4 mg daily; therapeutic, 5 to 10 mg daily.

Description : Yellow to orange-yellow crystalline powder; odour, slight; taste, slightly bitter.

Solubility : Slightly soluble in *water*; more soluble in *saline solution* and in a 1.0 per cent w/v solution of *urea*; sparingly soluble in *alcohol*; practically insoluble in *chloroform* and in *solvent ether*; soluble in dilute solutions of alkali hydroxides.

Standards : Riboflavine is 3,10-dihydro-7,8-dimethyl-10-[(2*S*, 3*S*, 4*R*)-2,3,4,5-tetrahydroxypentyl] benzopteridine-2,4-dione. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{17}H_{20}N_4O_6$, calculated with reference to the dried substance.

Identification : Dissolve about 1 mg in 100 ml of *water*. The solution has a pale greenish-yellow colour by transmitted light, and by reflected light has an intense yellowish-green fluorescence which disappears on addition of mineral acids or alkalis.

pH : Between 5.5 and 7.2, determined in a saturated solution, Appendix 5.10.

Specific optical rotation : Between −115° and −140° determined at 20° on a 0.5 per cent w/v solution in a mixture of 1.5 ml of carbon dioxide-free *0.1N alcoholic potassium hydroxide*, and sufficient freshly boiled and cooled *water* to produce 10 ml, within 30 minutes after preparing the solution, Appendix 5.12.

Light absorption : The solution prepared for the **Assay** described below, diluted with an equal volume of *water*, exhibits maxima at about 223 nm, 267 nm, 375 nm and 444 nm. The ratio of the *extinction* at 375 nm to that at 267 nm is between 0.31 and 0.33 and that of the *extinction* at 444 nm to that at 267 nm is between 0.36 and 0.39, Appendix 5.15 A.

Lumiflavin : Shake 25 mg with 10 ml of *alcohol-free chloroform* for five minutes and filter. *Extinction* of a 1-cm layer of the filtrate at 440 nm, using *alcohol-free chloroform* as the blank, does not exceed 0.025, Appendix 5.15 A.

NOTE – Prepare alcohol-free chloroform just prior to use.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.5 per cent, determined on 0.10 g by drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the assay in subdued light. Weigh accurately about 75 mg, add 150 ml of *water* and 2 ml of *glacial acetic acid* and heat on a water-bath with frequent agitation until the riboflavine is dissolved. Cool, and dilute with *water* to 1000.0 ml. To 10.0 ml of the solution, add 3.5 ml of *0.1M sodium acetate* and dilute with *water* to 50.0 ml. Measure the *extinction* of the resulting solution at the maximum at about 444 nm, Appendix 5.15 A. Calculate the content of $C_{17}H_{20}N_4O_6$, taking 323 as the value of E(1 per cent, 1-cm) at the maximum at about 444 nm.

Storage : Store in tightly-closed, light-resistant containers. Solutions, especially in the presence of alkali, deteriorate on exposure to light.

Riboflavine Tablets

Vitamin B_2 Tablets; Lactoflavin Tablets

Category : B-group Vitamin.

Dose : Riboflavine. Prophylactic, 1 to 4 mg daily; therapeutic, 5 to 10 mg daily.

Usual strengths : 2 mg; 5 mg.

Standards : Riboflavine Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of Riboflavine, $C_{17}H_{20}N_4O_6$.

Identification : Shake a quantity of the powdered tablets equivalent to about 1 mg of Riboflavine with 100 ml of *water* and filter; the filtrate complies with the **Identification** test described under Riboflavine.

Uniformity of content : Powder one tablet, add a mixture of 2.5 ml of *acetic acid* and 50 ml of *water* and heat on a water-bath for one hour with occasional stirring. Dilute with 50 ml of *water*, and 15 ml of *N sodium hydroxide* with continuous stirring and add sufficient *water* to produce a solution containing 10 µg of riboflavine per ml. Carry out the **Assay** on the resulting solution, beginning at the words "Measure the *extinction* . . . .". Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 and 110 per cent of the average, except that for one tablet the content may be between 85 and 115 per cent of the average.

Other requirements : Comply with the requirements stated under Tablets.

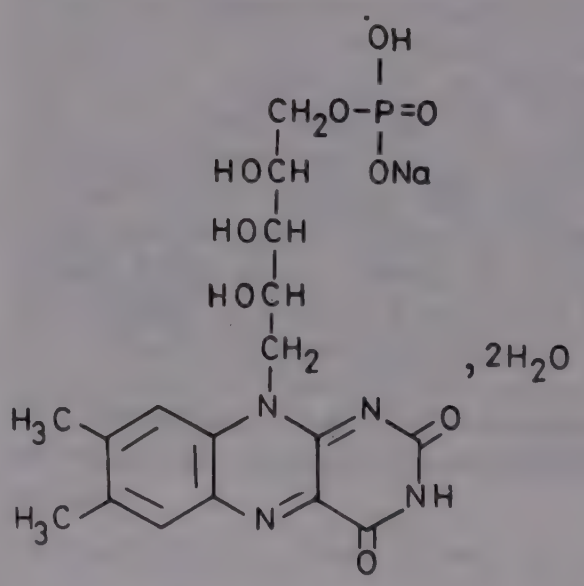
Assay : Carry out the assay in subdued light.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 10 mg of Riboflavine, add a mixture of 5 ml of *glacial acetic acid* and 100 ml of *water* and heat on a water-bath for one hour with occasional shaking. Dilute with *water*, cool, add 30 ml of *N sodium hydroxide* with continuous stirring, and sufficient *water* to produce 1000 ml; mix and filter, discarding the first portion of filtrate. Measure the *extinction* of a 1-cm layer of the filtrate at the maximum at about 444 nm, Appendix 5.15 A. Calculate the content of $C_{17}H_{20}N_4O_6$, taking 323 as the value of E(1 per cent, 1-cm) at the maximum at about 444 nm.

Storage : Store in tightly-closed, light-resistant containers.

Riboflavine Phosphate Sodium

Riboflavine-5-phosphate (Sodium salt); Vitamin B_2 Phosphate



$C_{17}H_{20}N_4NaO_9P,2H_2O$ Mol. Wt. 514.37

Category : B-group Vitamin.

Dose : Prophylactic; the equivalent of 1 to 3 mg of riboflavine, daily; therapeutic; the equivalent of 5 to 10 mg of riboflavine, daily.

NOTE – 1.4 g of riboflavine phosphate sodium is approximately equivalent to 1 g of riboflavine.

Description : Yellow to orange-yellow, crystalline powder; odourless or almost odourless; taste, slightly bitter; hygroscopic.

Solubility : Soluble in *water*, very slightly soluble in *alcohol*; insoluble in *solvent ether* and in *chloroform*.

Standards : Riboflavine Phosphate Sodium is the dihydrate of the sodium salt of Riboflavine-5-phosphate. It contains not less than 73.0 per cent and not more than the equivalent of 77.0 per cent of riboflavine, $C_{17}H_{20}N_4O_6$, calculated with reference to the dried substance.

Identification : (A) The light absorption, in the range 230 to 460 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *buffer solution, pH 7.0*, exhibits maxima only at 266 nm; *extinction* at 266 nm, about 0.61, Appendix 5.15 A.

(B) To 1 ml of a 0.01 per cent w/v solution in *water* add 1 ml of *N sodium hydroxide*. Expose the solution to ultra-violet radiation for 30 minutes, add sufficient *acetic acid* to make the solution acidic to litmus and shake the mixture with 2 ml of *chloroform*. The chloroform layer exhibits yellow fluorescence.

(C) Dissolve 0.1 g in 1 ml of warm *5N hydrochloric acid*, add 10 ml of *alcohol*, cool in an ice-bath and allow to crystallise. Filter through a sintered glass crucible. The residue after washing with *solvent ether* and drying melts at about 200°, Appendix 5.11.

(D) Dissolve 0.5 g in 10 ml of *nitric acid*, evaporate to dryness on a water-bath and ignite the residue until carbon is removed. Dissolve the ignited residue in 5 ml of *water* and filter; the filtrate gives the reactions of *sodium* and of *phosphates*, Appendix 3.1.

Specific optical rotation : Between +38° and +42°, determined in 1.5 per cent w/v solution in *5N hydrochloric acid*, Appendix 5.12.

pH : Between 4.0 and 6.3, determined in a 2 per cent w/v solution, Appendix 5.10.

Lumiflavin : To 0.035 g add 10 ml of *alcohol-free chloroform*, shake for 5 minutes and filter; the colour of the filtrate is not deeper than that of a solution prepared by diluting 3.0 ml of *0.1N potassium dichromate* to 1000 ml with *water*.

Free phosphate : Not more than 1.0 per cent, calculated as PO_4 and determined by the following method: Weigh accurately about 0.1 g and dissolve in sufficient *water* to produce 100.0 ml. Pipette 10 ml into a 25-ml volumetric flask, add 5.0 ml of *buffered copper sulphate solution, pH 4.0*, 2.0 ml of *dilute ammonium molybdate solution*, 1.0 ml of *strong methylaminophenol* with *sulphite solution*, and 1.0 ml of *perchloric acid solution*, shaking well for thirty seconds after each addition. Dilute to volume with *water* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 860 nm, Appendix 5.15 A, using a blank prepared in the same manner with 10 ml of *water* in place of 10 ml of the solution of the substance being examined. Calculate the content of free phosphate from the *extinction* obtained by carrying out the determination simultaneously on 9.5 ml of *water* and 0.5 ml of a standard phosphate solution prepared in the following manner: Weigh accurately 0.1433 g of *potassium dihydrogen phosphate* and dissolve in sufficient *water* to produce 1000.0 ml (each ml of standard phosphate solution contains 100 µg of phosphate, PO_4).

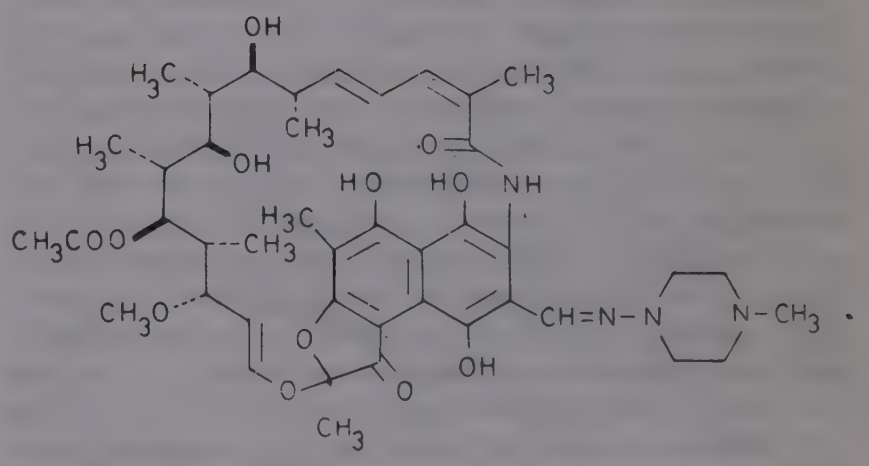
Loss on drying : Not more than 8.0 per cent, determined on 1 g by drying "in vacuo at 100°" for five hours, Appendix 5.8.

Assay : Carry out the assay in subdued light. Weigh accurately about 0.1 g, dissolve in 150 ml of *water*, add 2 ml of *glacial acetic acid* and dilute to 1000.0 ml with *water*. To 10.0 ml add 3.5 ml of *0.1N sodium acetate*, dilute to 50.0 ml with *water* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 444 nm, Appendix 5.15 A. Calculate the content of $C_{17}H_{20}N_4O_6$, taking 323 as the value of E(1 per cent, 1-cm) at the maximum at about 444 nm.

Storage : Store in well-closed, light-resistant containers.

Rifampicin

Rifampin



$C_{43}H_{58}N_4O_{12}$ Mol. Wt. 822.95

Category : Antibacterial.

Dose : 450 to 600 mg.

Description : Brick-red to reddish-brown, crystalline powder; odourless.

Solubility : Very slightly soluble in *water*; freely soluble in *chloroform*; soluble in *methyl alcohol*; slightly soluble in *alcohol* and in *solvent ether*.

Standards : Rifampicin is (12*Z*, 14*E*, 24*E*)-(2*S*, 16*S*, 17*S*, 18*R*, 19*R*, 20*R*, 21*S*, 22*R*, 23*S*)-1,2-dihydro-5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1-

yliminomethyl)-1, 11-dioxo-2, 7-(epoxypentadeca-1, 11, 13-trienoimino)naphtho-[2, 1-*b*]furan-21-yl-acetate. It contains not less than 900 μg of $C_{43}H_{58}N_4O_{12}$ per mg, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *rifampicin R.S*, Appendix 5.15 B.

(B) Suspend 25 mg in 25 ml of *water*, shake for five minutes and filter. To 5 ml of the filtrate add 1 ml of a 10 per cent w/v solution of *ammonium persulphate* in *buffer solution, pH 7.4* and shake for a few minutes. The colour turns from orange-yellow to violet-red without the formation of any precipitate.

Light absorption : Dissolve 0.1 g in sufficient *methyl alcohol* to produce 100.0 ml, dilute 2.0 ml to 100.0 ml with *buffer solution, pH 7.4*. The light absorption of the resulting solution, in the range 220 to 500 nm, exhibits four absorption maxima, at 237 nm, 254 nm, 334 nm and 475 nm, the ratio of the *extinction* of a 1-cm layer at the maximum at 334 nm to that at 475 nm is about 1.75, Appendix 5.15 A.

pH : Between 4.5 and 6.5, determined in a 1 per cent w/v suspension in *carbon dioxide-free water*, Appendix 5.10.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 85 volumes of *chloroform* and 15 volumes of *methyl alcohol* as the mobile phase and allowing the solvent front to ascend 12 cm above the line of application. Prepare the plate by spraying it with a solution containing 0.774 g of *citric acid* in sufficient *0.2 M disodium hydrogen phosphate* to produce 100 ml and then drying at 100° for one hour. Apply separately to the plate 5 μl of each of two freshly prepared solutions in *chloroform* containing (1) 4.0 per cent w/v of the substance being examined and (2) 0.08 per cent w/v of the substance being examined. After removal of the plate allow it to dry in air at room temperature; any coloured spot other than the principal spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Undue toxicity : complies with the test described under Cephalexin, the dose being 1.0 ml of suspension containing 6 mg of Rifampicin in a 1 per cent w/v solution of *methylcellulose (4000 cps)*.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 2.0 per cent, determined on 0.5 g by drying "in vacuo at 80°" for four hours, Appendix 5.8.

Assay : Use either of the following methods; however, the result obtained from the microbiological assay shall be official:

(1) Weigh accurately about 0.1 g and dissolve in sufficient *methyl alcohol* to produce 100.0 ml; dilute 2.0 ml to 100.0 ml with *buffer solution, pH 7.4* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 475 nm, Appendix 5.15 A. Calculate the content of $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of E(1 per cent, 1-cm) at the maximum at about 475 nm.

(2) Weigh accurately about 0.1 g and dissolve in sufficient *methyl alcohol* to give a solution containing about 1.0 mg of Rifampicin per ml. Dilute a suitable aliquot of this solution with *buffer solution No.1*, Appendix 4.1, Table 2, to give a solution containing about 5.0 μg of Rifampicin per ml. Carry out the *microbiological assay of antibiotics, Method A*, Appendix 4.1, and express the results in μg of rifampicin per mg.

Storage : Store in tightly-closed, light-resistant containers in an atmosphere of nitrogen and at a temperature not exceeding 15°.

Labelling : The label on the container states (1) the date after which the contents are not intended to be used; (2) the storage conditions.

Rifampicin Capsules

Rifampin Capsules

Category : Antibacterial.

Dose : Rifampicin, 450 to 600 mg.

Usual strengths : 150 mg; 300 mg.

Standards : Rifampicin Capsules contain not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of Rifampicin, $C_{43}H_{58}N_4O_{12}$.

Identification : The contents of the capsules comply with **Identification** tests (A) and (B) described under Rifampicin.

Related substances : Comply with the test described under Rifampicin, using as solution (1) a solution freshly prepared by shaking a quantity of the contents of the capsules equivalent to 0.4 g of Rifampicin with 10 ml of *chloroform* and filtering. Solution (2) is prepared by diluting 1 ml of solution (1) with sufficient *chloroform* to produce 50 ml.

Other requirements : Comply with the requirements stated under Capsules.

Loss on drying : Not more than 3.0 per cent, determined on 0.5 g of the contents of the capsules, by drying "in vacuo at 80°" for four hours, Appendix 5.8.

Assay : Use either of the following methods; however, the result obtained from the microbiological assay shall be official:

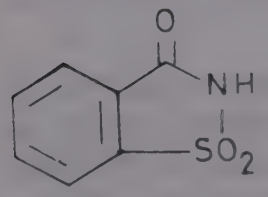
(1) Weigh accurately a quantity of the mixed contents of 20 capsules, equivalent to 0.1 g of Rifampicin, add sufficient *methyl alcohol* to produce 100.0 ml. Shake for ten minutes and filter. Dilute 2.0 ml of the filtrate to 100.0 ml with *buffer solution, pH 7.4* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 476 nm, Appendix 5.15 A. Calculate the content of $C_{43}H_{58}N_4O_{12}$, taking 188 as the value of E(1 per cent, 1-cm) at the maximum at about 476 nm.

(2) Weigh accurately a quantity of the mixed contents of 20 capsules, equivalent to 0.1 g of Rifampicin and complete **Assay** (2) described under Rifampicin, beginning at the words "dissolved in sufficient *methyl alcohol* . . . . :"

Storage : Store in tightly-closed, light-resistant containers, in a cool place.

Labelling : The label on the container states (1) the date after which the contents are not intended to be used; (2) the storage conditions.

Saccharin



$C_7H_5NO_3S$ Mol. Wt. 183.18

Category : Pharmaceutical aid (sweetening agent).

Description : White crystals or white crystalline powder; odourless or with a faint, aromatic odour; taste, intensely sweet, even in dilute solution.

Solubility : Slightly soluble in *water*, in *chloroform*, and in *solvent ether*; soluble in *acetone* and in boiling *water*; readily soluble in *dilute ammonia solution*, in solutions of alkali hydroxides and in solutions of alkali carbonates with the evolution of carbon dioxide.

Standards : Saccharin is 1,2-benzisothiazolin-3-one 1,1-dioxide. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_7H_5NO_3S$, calculated with reference to the dried substance.

Identification : (A) Mix 20 mg with 40 mg of *resorcinol*, add 10 drops of *sulphuric acid*, and heat over a small flame until it assumes a dark green colour; allow to cool and add 10 ml of *water* and an excess of *sodium hydroxide solution*; a fluorescent green liquid is produced.

(B) Dissolve 0.1 g in 5 ml of a 10 per cent w/v solution of *sodium hydroxide*, evaporate to dryness, and gently fuse the residue over a small flame until ammonia is no longer evolved. Allow to cool, dissolve in 20 ml of *water*, neutralise the solution with *dilute hydrochloric acid*, and filter; on the addition of a drop of *ferric chloride test-solution* a violet colour is produced.

(C) A saturated solution is acid to *litmus solution*.

Melting range : Between 226° and 230°, Appendix 5.11.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Readily carbonisable substances : Dissolve 0.2 g in 5 ml of *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4) and keep at a temperature of 48° to 50° for ten minutes; the solution is not more coloured than a mixture of 0.1 ml of *cobalt chloride C.S.*, 0.4 ml of *ferric chloride C.S.*, 0.1 ml of *copper sulphate C.S.*, and 4.4 ml of *water*.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 100 volumes of *chloroform*, 50 volumes of *methyl alcohol* and 11.5 volumes of *strong ammonia solution* as the mobile phase. Apply separately to the plate 5 µl of each of four solutions. For solution (1) dissolve 2.0 g of the substance being examined in 10 ml of a 10 per cent w/v solution of *sodium bicarbonate* and mix well with 12.5 g of white diatomaceous filter-aid (Celite 545 filtration grade is suitable). Transfer to a 25mm × 250mm chromatographic tube having a fritted glass disc and stop-cock at the bottom. Pack the contents of the tube by tapping on a padded surface and then by tamping firmly from the top. Elute with *methylene chloride* so that 50 ml of eluate is obtained in about 30 minutes. Evaporate the eluate to dryness and dissolve the residue in 4.0 ml of *acetone*. Solution (2) is a 0.010 per cent w/v solution of *toluene-o-sulphonamide R.S.* in *acetone*; solution (3) is a 0.50 per cent w/v solution of the substance being examined in *methyl alcohol* and solution (4) is a 0.0050 per cent w/v solution of *4-sulphamoylbenzoic acid* in *acetone*. After removal of the plate, dry it in a current of warm air, heat at 105° for five minutes, and spray the hot plate with *sodium hypochlorite solution* diluted with *water* to contain 0.5 per cent w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a faint blue colour with a drop of a 0.5 per cent w/v solution of *potassium iodide* in *starch solution* containing 1 per cent v/v of *glacial acetic acid*; avoid prolonged exposure to cold air. Spray the plate with the same mixture. The spot in the chromatogram obtained with solution (2) is more intense than any corresponding spot in the chromatogram obtained with solution (1) and

the spot in the chromatogram obtained with solution (4) is more intense than any corresponding spot in the chromatogram obtained with solution (3).

Benzoic and salicylic acids : To 10 ml of a hot saturated solution add *ferric chloride solution*, dropwise; no precipitate or violet colour is seen in the liquid.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

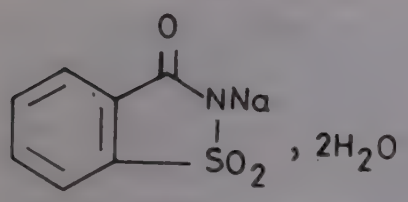
Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g, dissolve in 75 ml of hot *water*, cool quickly and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01832 g of $C_7H_5NO_3S$.

Storage : Store in well-closed containers.

Saccharin Sodium

Soluble Saccharin



$C\text{-}H_4NNaO_3S,2H_2O$ Mol. Wt. 241.19

Category : Pharmaceutical aid (sweetening agent).

Description : White crystalline powder; odourless, or with a faint aromatic odour; taste, intensely sweet, even in dilute solution. It is efflorescent.

Solubility : Freely soluble in *water*; sparingly soluble in *alcohol*.

Standards : Saccharin Sodium is the dihydrate of the sodium salt of 1,2-benzisothiazolin-3-one 1,1-dioxide. It contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of $C\text{-}H_4NNaO_3S$, calculated with reference to the anhydrous substance.

Identification : (A) Complies with **Identification** tests (A) and (B) described under Saccharin.

(B) The light absorption in the range 230 to 350 nm, of a 1-cm layer of a 0.01 per cent w/v solution in *0.1N hydrochloric acid* exhibits maxima at 275 nm and at 283 nm; *extinction* at 275 nm, about 0.55, at 283 nm, about 0.42. Appendix 5.15 A.

(C) The residue left on incineration gives the reactions of *sodium*, and of *sulphates*, Appendix 3.1.

Melting range of isolated saccharin : Dissolve 1 g in 10 ml of *water* and add 1 ml of *hydrochloric acid*; a crystalline precipitate is produced which when washed and dried has a melting range between 226° and 230°, Appendix 5.11.

Free acid or Alkali : To a solution of 1 g in *carbon dioxide-free water* add 5 ml of *0.01N sulphuric acid*, boil, cool, and titrate with *0.01N sodium hydroxide*, using *phenolphthalein solution* as indicator; not less than 4.5 ml and not more than 5.5 ml of *0.01N sodium hydroxide* is required.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined by Method A on a solution prepared in the following manner: Dissolve 2 g in 48 ml of *water*, add 2 ml of *1N hydrochloric acid*, mix well, and rub the inner wall of the vessel with a glass rod until crystallisation begins. Allow the solution to stand for one hour, then filter through a dry filter, rejecting the first 10 ml of the filtrate; carry out the test on the next 25 ml of the filtrate, Appendix 3.2.4.

Related substances : Complies with the test described under Saccharin, using in solution (1) 2.6 g of the substance being examined, and for solution (2) a 0.005 per cent w/v solution of *toluene-o-sulphonamide R.S.* in *acetone*.

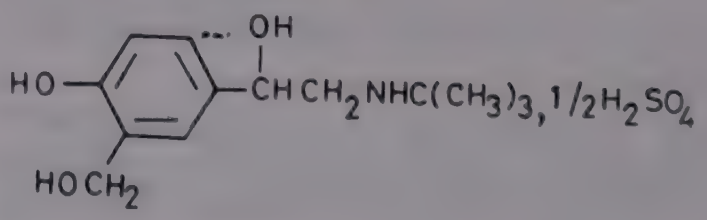
Water : Between 12.0 per cent and 16.0 per cent w/w, Appendix 3.3.25.

Assay : Weigh accurately about 0.3 g and dissolve in 20 ml of *glacial acetic acid*. Add a few drops of *crystal-violet solution* and titrate with *0.1N perchloric acid*. Perform a blank titration and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02052 g of $C_7H_4NNaO_3S$.

Storage : Store in well-closed containers.

Salbutamol Sulphate

Albuterol Sulphate



$C_{13}H_{21}NO_3, \frac{1}{2}H_2SO_4$ Mol. Wt. 288.35

Category : Adrenergic (bronchodilator).

Dose : The equivalent of 6 to 16 mg of salbutamol, daily, in divided doses.

Description : White or almost white powder; odourless; taste, slightly bitter.

Solubility : Freely soluble in *water*; slightly soluble in *alcohol*; in *chloroform*, and in *solvent ether*.

Standards : Salbutamol Sulphate is the hemisulphate of 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*t*-butylamino)ethanol. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{13}H_{21}NO_3,\frac{1}{2}H_2SO_4$, calculated with reference to the dried substance.

Identification : (A) Dissolve 10 mg in 50 ml of a 2 per cent w/v solution of *borax*, add 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide*, and 10 ml of *chloroform*, shake, and allow to separate; an orange-red colour develops in the chloroform layer.

(B) To 2 ml of a 1 per cent w/v solution, add 2 drops of *ferric chloride test-solution*; a reddish-orange colour develops which does not change on the addition of *sodium bicarbonate solution*.

(C) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.008 per cent w/v solution in *0.1N hydrochloric acid* exhibits a maximum only at 276 nm; *extinction* at 276 nm, about 0.46, Appendix 5.15 A.

(D) A solution (1 in 10) gives the reactions of *sulphates*, Appendix 3.1.

Foreign substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 4 volumes of *strong ammonia solution*, 16 volumes of *water*, 30 volumes of *isopropyl alcohol* and 50 volumes of *ethylacetate* as the mobile phase. Apply separately to the plate 10 µl each of two solutions in *methyl alcohol* containing (1) 2.0 per cent w/v of the substance being tested and (2) a 0.01 per cent w/v of *1-(4-hydroxy-3-methylphenyl)-2-(t-butylamino) ethanol R.S.* After removal of the plate, allow it to dry in air until the odour of the solvent is not detectable, place it for a few minutes in an atmosphere saturated with *diethylamine*, and spray with *diazotised nitroaniline solution*. Any spot in the chromatogram obtained with solution (1), other than the principal spot, is not more intense than the spot in the chromatogram obtained with solution (2).

Boron : Not more than 50 parts per million, determined by the following method : To 50 mg add 5 ml of a 3 per cent w/v solution of an equimolecular mixture of *anhydrous sodium carbonate* and *potassium carbonate*, evaporate to dryness on a water-bath and dry at 120°. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool and add 0.5 ml of *water* and 3.0 ml of a freshly prepared 0.125 per cent w/v solution of *curcumin* in *glacial acetic acid*. Warm gently to effect solution, allow to cool and add 3.0 ml of a mixture prepared by adding 5 ml of *sulphuric acid*, slowly, with stirring, to 5 ml of *glacial acetic acid*. Mix, and allow to stand for thirty minutes. Add sufficient *alcohol* to produce 100.0 ml, filter, and measure the *extinction* of a 1-cm layer of the filtrate at the maximum at about 555 nm, Appendix 5.15 A. Calculate the content of boron from a reference curve prepared from the *extinctions* obtained by treating suitable aliquots of a solution of *boric acid* in the same manner.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent determined on 1.0 g by drying "in vacuo at 100°", Appendix 5.8.

Assay : Weigh accurately about 0.9 g and dissolve in 50 ml of *glacial acetic acid* by warming. Cool, protecting from moisture, add 0.15 ml of *oracet blue B solution* and titrate with *0.1N perchloric acid* to the purple end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.05767 g of $C_{13}H_{21}NO_3,\frac{1}{2}H_2SO_4$.

Storage : Store in well-closed, light-resistant containers.

Salbutamol Tablets

Category : Adrenergic (bronchodilator).

Dose : The equivalent to 6 to 16 mg of Salbutamol daily, in divided doses.

Usual strengths : 2 mg and 4 mg of Salbutamol.

Standards : Salbutamol Tablets contain an amount of Salbutamol Sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Salbutamol, $C_{13}H_{21}NO_3$.

Identification : (A) Shake a quantity of the powdered tablets equivalent to 2.0 mg of Salbutamol with 50 ml of a 2 per cent w/v solution of *borax*, add 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide*, and 10 ml of *chloroform*, shake, and allow to separate; an orange-red colour develops in the chloroform layer.

(B) Shake a quantity of the powdered tablets equivalent to 4 mg of Salbutamol with 10 ml of *water* and filter; the filtrate gives the reactions of *sulphates*, Appendix 3.1.

Uniformity of content : Stir for thirty minutes a volume of about 100 ml of a strongly basic anion exchange resin with 500 ml of a 5 per cent w/v solution of *sodium hydroxide*, discard the liquid, wash with successive quantities of *water* until the washings are neutral to *litmus solution*, stir for thirty minutes with 250 ml of 5 per cent w/v solution of *sulphuric acid*, and wash with successive

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quantities of *water* until the pH of the washings is not below 5.0. Pack the resin into a tube to form a column about 3 cm high. Powder one tablet and shake the powder for thirty minutes with a mixture of 5 ml of *0.1N hydrochloric acid* and 15 ml of *water*. Filter, wash the filter with 10 ml of *water*, and pass through the column the combined filtrate and washings, add sufficient *water* to produce 50 ml. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 276 nm, Appendix 5.15 A. Calculate the content of $C_{13}H_{21}NO_3$, taking 71 as the value of E(1 per cent, 1-cm) at the maximum at about 276 nm.

Repeat the operation with a further nine tablets, using fresh ion-exchange resin for each tablet. Calculate the average content of the ten tablets. The content of each tablet is between 90 per cent and 110 per cent of the average except that for one tablet the content may be between 85 per cent and 115 per cent of the average.

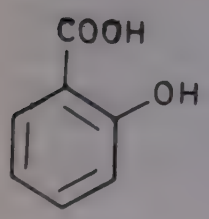
Other requirements : Comply with the requirements stated under Tablets.

Assay : Prepare a column about 3 cm high of a strong basic anion exchange resin as directed under **Uniformity of content**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 2 mg of Salbutamol and shake for thirty minutes with a mixture of 5 ml of *0.1N hydrochloric acid* and 15 ml of *water*. Proceed as directed under **Uniformity of content**, beginning with "Filter, wash the filter....".

Storage : Store in well-closed, light-resistant containers.

Labelling : The label on the container states the strength in terms of the equivalent amount of Salbutamol.

Salicylic Acid



$C_7H_6O_3$ Mol. Wt. 138.12

Category : Keratolytic.

Description : Colourless or almost colourless feathery crystals, or a white powder; almost odourless; taste, sweetish and acrid.

Solubility : Freely soluble in *alcohol* and in *solvent ether*; soluble in boiling *water*; sparingly soluble in *chloroform*; slightly soluble in *water*.

Standards : Salicylic Acid is 2-hydroxybenzoic acid. It contains not less than 99.5 per cent and not more than the equivalent of 101.0 per cent of $C_7H_6O_3$, calculated with reference to the dried substance.

Identification : It gives the reactions of *salicylates*, Appendix 3.1.

Melting range : Between 158° and 161°, Appendix 5.11.

Readily carbonisable substances : Dissolve 0.5 g in 5 ml of *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4); the solution is not more coloured than a mixture of 0.1 ml of *cobalt chloride C.S.*, 0.5 ml of *ferric chloride C.S.*, 0.1 ml of *copper sulphate C.S.* and 4.2 ml of *water*.

Heavy metals : Dissolve 1 g in 25 ml of *acetone* and add 2 ml of *water* and 10 ml of *hydrogen sulphide solution*. Any colour produced is not darker than that of a control made with 25 ml of *acetone*, 2 ml of *standard lead solution*, and 10 ml of *hydrogen sulphide solution*.

Iron : Boil 12.0 g with 14 ml of *dilute ammonia solution* and 35 ml of *water*. Cool, and adjust the pH to 5.0 to 6.0 by the dropwise addition of *dilute ammonia solution* or *dilute sulphuric acid*. Any pink colour produced is not deeper than that obtained by boiling 2.0 g with 1 ml of *standard iron solution*, 2 ml of *dilute ammonia solution* and 45 ml of *water*, adjusting the pH to 5.0 to 6.0 and diluting to 50 ml with *water*.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying over *silica gel* for three hours, Appendix 5.8.

Assay : Weigh accurately about 2 g and dissolve in 15 ml of warm *alcohol* which has previously been neutralised to *phenol red solution*, add 20 ml of *water*, and titrate with *0.5N sodium hydroxide*, using *phenol red solution* as indicator. Each ml of *0.5N sodium hydroxide* is equivalent to 0.06905 g of $C_7H_6O_3$.

Storage : Store in well-closed containers.

Schick Test Toxin

Diphtheria Toxin for Schick Test

Category : Diagnostic aid (dermal reactivity indicator).

Dose : By intradermal injection, 1 ml.

Description : Clear, colourless or very pale straw-coloured liquid.

Standards : Schick Test Toxin is the preparation used in the Schick Test to determine susceptibility to diphtheria. It is prepared from a sterile filtrate of a culture in liquid medium of a toxigenic strain of the diphtheria bacillus (*Corynebacterium diphtheriae*) and then purified. It is then diluted so that the test dose is contained in 0.1 ml or 0.2 ml, with a sterile diluent isotonic with blood and containing suitable bactericides and stabilisers. It is distributed into sterile containers which are sealed so as to exclude micro-organisms.

Identification : It causes a local reaction when injected into the skin of a healthy, white guinea-pig or rabbit that has not previously been treated with any material that will interfere with the test but fails to cause this reaction when mixed with sufficient quantity of diphtheria antitoxin.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Assay : The potency of one human dose (0.1 ml or 0.2 ml) is defined in terms of its combining power with diphtheria antitoxin and its erythrogenic activity measured in International Units of Schick test toxin.

(1) *Combining power*—Prepare two mixtures of the toxin such that in one mixture 1 test dose is mixed with 1/750 of 1 International Unit of diphtheria antitoxin and in the other 1 test dose is mixed with 1/1250 International Unit of diphtheria antitoxin. Allow both mixtures to stand at room temperature for thirty to sixty minutes and inject each, on separate flanks, into the depilated skin of a white guinea-pig weighing not less than 250 g or of a white rabbit that weighs not less than 2.5 kg, neither of which has been treated with any material that interferes with the test. Examine the animal two days after infection. No reaction occurs at the site infected with the mixture containing 1/750 of 1 International Unit of diphtheria antitoxin but at the site infected with the other mixture a reaction of the type known as "positive Schick reaction" occurs.

(2) Depilate two healthy, white guinea-pigs or rabbits that have not been treated with any material that will interfere with the test in such a way that each has an area of denuded skin sufficient for 16 injection sites, two columns of four sites on each flank. Prepare two solutions of the preparation to be examined and two solutions of the Standard Preparation such that for both preparations one solution contains toxin at one-fifth the concentration of the toxin in the other solution and both evoke erythematous lesions of opposite size when injected intradermally in 0.2 ml volumes into the depilated skins of the test animals.

Inject 0.2 ml of each of the four solutions so prepared, in turn, intradermally at each of four sites or each guinea-pig or rabbit, and distribute the four injections of each dilution among the sixteen available sites in each animal in a Latin square design.

Measure the longitudinal and transverse axes of the lesions at the injection sites two days after the injections have been made. Using for each lesion the geometric mean of the measurements, calculate by regression analysis the erythrogenic activity of one test (Human) dose in International Units of Schick test toxin. The estimated potency is not less than 0.5 and not more than 2.0 International Units.

Storage : Store at a temperature between 2° and 10°.

Labelling : The label on the container states (1) the volume of the test dose; (2) the total volume in the container; (3) the storage conditions; (4) the expiry date.

SCHICK CONTROL

Schick Test Control

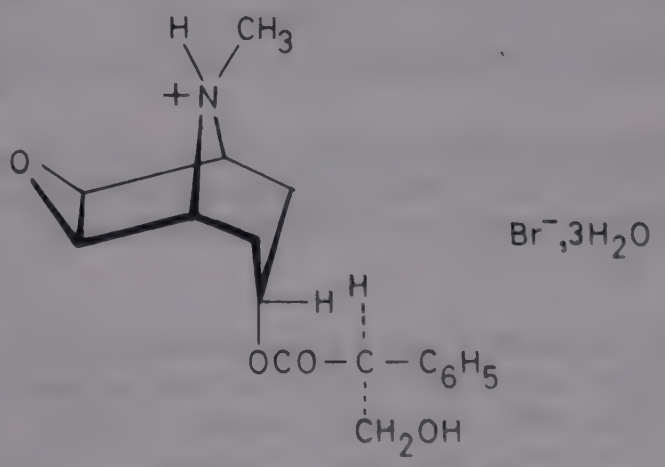
Standards : Schick Control is Schick Test Toxin which has been heated at a temperature not lower than 70° and not higher than 85° for not less than five minutes. It may be used in conjunction with Schick test toxin in order to exclude reactions due to non-specific substances. It must be prepared from the same batch of Schick Test Toxin as that with which it is to be used.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Storage : Store at a temperature between 2° and 10°.

Scopolamine Hydrobromide

Hyoscine Hydrobromide



$C_{17}H_{21}NO_4,HBr,3H_2O$ Mol. Wt. 438.31

Category : Anticholinergic.

Dose : By subcutaneous injection, 300 to 600 micrograms.

Description : White, crystalline powder, slightly efflorescent; odourless.

Solubility : Freely soluble in *water*; soluble in *alcohol*; practically insoluble in *solvent ether*, and in *chloroform*.

Standards : Scopolamine Hydrobromide is trihydrate of (1*S*,3*s*,5*R*,6*R*,7*S*)-6,7-epoxy-3-[(*S*)-tropoyloxy]-tropanium bromide. It contains not less than 98.5 per cent of $C_{17}H_{22}BrNO_4$, calculated with reference to the dried substance.

Identification : (A) To about 1 mg , add 4 drops of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 4 drops of a 3 per cent w/v solution of *potassium hydroxide* in *methyl alcohol*. A violet colour is produced.

(B) Dissolve a few mg in 5 ml of *water* and add 1 ml *potassium iodobismuthate solution*; an orange or orange-red precipitate is produced.

(C) A solution (1 in 20) gives the reactions of *bromides*, Appendix 3.1.

Melting range : Between 195° and 199°, with decomposition; Appendix 5.11.

Specific optical rotation : Between −24° and −27°, calculated with reference to the dried substance and determined in a 5 per cent w/v solution, Appendix 5.12.

pH : Between 4.0 and 5.5, determined in a 5 per cent w/v solution, Appendix 5.10.

Foreign alkaloids : Dissolve 0.5 g in 10 ml of freshly boiled and cooled *water*, add 3 drops of *dilute ammonia solution*. No permanent turbidity is produced.

Readily oxidisable substances : To 3 ml of a 5 per cent w/v solution add 12 ml of *water*, and 0.05 ml of *0.1N potassium permanganate*. The colour of the permanganate is not completely discharged within 5 minutes.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not less than 10.0 per cent and not more than 13.0 per cent, determined on 1.0 g by drying "in vacuo" for one hour followed by drying in an oven at 100° to 105°, Appendix 5.8.

Assay : Weigh accurately about 0.4 g and dissolve in 20 ml of *glacial acetic acid*. Add 10 ml of *mercuric acetate solution* and titrate with *0.1N perchloric acid*, determining the end-point potentiometrically. Each ml of *0.1N perchloric acid* is equivalent to 0.03843 g of $C_{17}H_{21}NO_4,HBr$.

Storage : Store in well-closed, light-resistant containers in a cool place.

Scopolamine Hydrobromide Tablets

Hyoscine Hydrobromide Tablets

Category : Anticholinergic.

Dose : Scopolamine Hydrobromide, 0.3 to 1.2 mg as a single dose.

Usual strengths : 0.4 mg; 0.6 mg.

Standards : Scopolamine Hydrobromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Scopolamine Hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$.

Identification : (A) Shake a quantity of the powdered tablets equivalent to about 1 mg of Scopolamine Hydrobromide with 5 ml of *alcohol* for two minutes, filter and evaporate the filtrate to dryness on a water-bath. Cool, add 4 drops of *fuming nitric acid* and again evaporate to dryness on a water-bath; a yellow residue is obtained. Cool and add to the residue 2 ml of *acetone* and 4 drops of 3 per cent w/v solution of *potassium hydroxide* in *methyl alcohol*; a deep violet colour is produced.

(B) Shake a quantity of the powdered tablets equivalent to 1 mg of Scopolamine Hydrobromide with 10 ml of *water* and filter; the filtrate gives the reactions of *bromides*, Appendix 3.1.

Uniformity of content : Carry out the **Assay** on one tablet using for solution (2) the following: Powder one tablet and shake in a centrifuge tube with 5.0 ml of *water*; centrifuge, pipette 2.0 ml of the supernatant liquid into a separator; add 2.0 ml of *buffer solution, pH 9.0*, shake vigorously with 5 ml of *methylene chloride*, allow the layers to separate and filter the methylene chloride extract through about 2 g of *anhydrous sodium sulphate*. Evaporate the filtrate under reduced pressure almost to dryness and dissolve the residue in 0.8 ml of *methylene chloride*. Solution (3) is prepared by extracting 2.0 ml of the supernatant liquid obtained while preparing solution (2), 0.8 ml of a freshly prepared 0.125 per cent w/v solution of *atropine sulphate R.S.* (internal standard) and 2.0 ml of *buffer solution, pH 9.0* with 5 ml of *methylene chloride* and proceeding as directed for solution (2). Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablet.

Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 and 110 per cent of the average except that for one tablet the content may be between 85 and 115 per cent of the average.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Carry out the method for *gas-liquid chromatography*, Appendix 5.4.1, using the following solutions : For solution (1) shake vigorously 2.5 ml of a freshly prepared 0.025 per cent w/v solution of *atropine sulphate R.S.*, previously dried at 120° for four hours (internal standard), 2.5 ml of a freshly prepared 0.02 per cent w/v solution of *scopolamine hydrobromide R.S.*, previously dried at 105° for three hours, 5 ml of *buffer solution, pH 9.0* with 10 ml of *methylene chloride*. Allow the layers to separate and filter the methylene chloride, extract through about 2 g of *anhydrous sodium sulphate*. Evaporate the filtrate under reduced pressure to about 2 ml and dilute with *methylene chloride* to 5.0 ml. For solution (2) weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 1 mg of Scopolamine Hydrobromide and disperse in 5 ml of *buffer solution, pH 9.0*. Shake vigorously with 10 ml of *methylene chloride*, allow the layers to separate and filter the methylene chloride extract through about 2 g of *anhydrous sodium sulphate*. Evaporate the filtrate under reduced pressure to about 2 ml and dilute with *methylene chloride* to 5.0 ml. Solution (3) is prepared in a similar manner to solution (2) using in addition, 5.0 ml of a freshly prepared 0.03 per cent w/v solution of the internal standard and extracting with 10 ml of *methylene chloride*.

The chromatographic procedure may be carried out using (a) a glass column 1.5 m long and 0.4 cm in internal diameter packed with 3 per cent w/w of phenylmethyl silicone fluid on acid-washed silanised, diatomaceous earth (100 to 120 mesh) maintained at a temperature of 230°; (b) helium as the carrier gas, and (c) flame ionisation detector. Calculate the content of $C_{17}H_{21}NO_4, HBr, 3H_2O$ from the declared content of $C_{17}H_{21}NO_4, HBr, 3H_2O$ in the *scopolamine hydrobromide R.S.*

Storage : Store in tightly-closed, light-resistant containers.

Scopolamine Injection

Hyoscine Injection

Category : Anticholinergic.

Dose : Scopolamine Hydrobromide, by subcutaneous injection, 0.3 to 0.6 mg.

Usual strength : 0.4 mg in 1 ml.

Standards : Scopolamine Injection is a sterile solution of Scopolamine Hydrobromide in Water for Injection. It contains not less than 90.0 per cent and not more than 110.0 per cent of the stated content of $C_{17}H_{21}NO_4, HBr, 3H_2O$.

Identification : (A) Dilute a volume equivalent to about 1 mg of Scopolamine Hydrobromide with *dilute ammonia solution* till the solution is alkaline to *litmus paper* and extract the base with *chloroform*. Evaporate the filtered chloroform solution in a porcelain dish on a water-bath to dryness; the residue complies with the **Identification** test (A) described under Scopolamine Hydrobromide.

(B) To 5 ml add *silver nitrate solution*; a yellowish-white precipitate insoluble in *nitric acid* but soluble in *dilute ammonia solution* is formed.

pH : Between 4.0 and 6.0, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

Assay : Carry out the method for *gas-liquid chromatography*, Appendix 5.4.1, using the following solutions: For solution (1) to 15 ml of a 0.33 per cent w/v solution of *scopolamine hydrobromide R.S.* add 1 ml of a 0.30 per cent w/v solution of *atropine sulphate R.S.* (internal standard) in *methyl alcohol* (solution A) and 1 ml of *dilute ammonia solution*. Extract with two quantities, each of 10 ml, of *chloroform*, shake the combined extracts with 2 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *methylene chloride*. To 1 ml of this solution add 0.2 ml of a mixture of 4 volumes of *NO-bis-(trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for thirty minutes. Solution (2) is prepared in the same manner as solution (3) but omitting the addition of solution (A). For solution (3) dilute a volume of the injection equivalent to 5 mg of Scopolamine Hydrobromide to 15 ml with *water* and proceed as described under solution (1) beginning at the words "add 1 ml of a 0.30 per cent w/v solution .....".

The chromatographic procedure may be carried out using (a) a glass column 1.5 cm long and 0.4 cm in internal diameter packed with 3 per cent w/w of phenylmethyl silicone fluid (50 per cent phenyl), maintained at 230°, (b) *nitrogen* as the carrier gas and (c) a flame ionisation detector. Calculate the content of $C_{17}H_{21}NO_4, HBr, 3H_2O$ from the declared content of $C_{17}H_{21}No_4, HBr, 3H_2O$ in the *scopolamine hydrobromide R.S.*

Storage : Store in single-dose or multiple-dose, light-resistant containers.

Senna Fruit

Senna Pod

Category : Cathartic.

Dose : 0.6 to 2.0 g.

Standards : Senna Fruit consists of the dried fruits of *Cassia acutifolia* Delile, known in commerce as Alexandrian Senna Pods and of *Cassia angustifolia* Vahl, known in commerce as Tinnevally Senna Pods. It contains not less than 1.5 per cent of hydroxyanthracene derivatives, calculated as sennoside B.

Description : Flattened reniform pods, brownish-yellow at the edges, dark brown in the central area, about 40 to 50 mm long (Alexandrian senna pods) or about 35 to 60 mm long (Tinnevally senna pods), and at least 20 mm wide (Alexandrian senna pods) or 14 to 18 mm wide (Tinnevally senna pods). At one end is a stylar point and at the other a short stalk. The pods contain 5 to 8 flattened and obovate seeds, green to pale-brown, with a continuous network of prominent ridges on the testa. Odour and taste, slight.

Examined under a microscope the pods present an epicarp with strongly cuticularised isodiametrical cells, occasional anomocytic or paracytic stomata, and very few conical, unicellular and warty hairs; hypodermis with collenchymatous cells; mesocarp with parenchymatous tissue and a layer of prisms of calcium oxalate; endocarp consisting of thick-walled and interlacing fibres.

The seeds present a sub-epidermal layer of palisade cells with thick outer walls. The endosperm has polyhedral cells with mucilaginous walls.

The powder is brown in colour and consists of epicarp with polygonal cells and a small number of conical warty hairs and occasional anomocytic or paracytic stomata. Fibres in two crossed layers accompanied by a crystal sheath of calcium oxalate. Characteristic palisade cells in the seed and stratified cells in the endosperm; clusters and prisms of calcium oxalate.

Identification : To 25 mg of the powdered drug add 50 ml of *water* and 2 ml of *hydrochloric acid*; heat on a water-bath for fifteen minutes, allow to cool and shake with 40 ml of *solvent ether*. Separate the ether extract and dry over *anhydrous sodium sulphate*. Evaporate 5 ml to dryness, cool and add 5 ml of *dilute ammonia solution*. A yellow or orange colour develops. Heat on a water-bath for two minutes; a reddish-white colour develops.

Foreign organic matter : Not more than 1.0 per cent, Appendix 3.3.22.

Sulphated ash : Not more than 6.0 per cent, Appendix 3.2.7.

Acid-insoluble ash : Not more than 2.0 per cent, Appendix 3.3.22.

Assay : Protect the solutions from light throughout the assay. Weigh accurately about 0.3 g of the powdered drug, add 30 ml of *water*, mix and weigh again. Place in a water-bath and heat under a reflux condenser for fifteen minutes. Cool, weigh and adjust the weight with *water*. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separator, add 0.1 ml of *dilute hydrochloric acid* and shake with three quantities, each of 15 ml, of *chloroform*. Discard the chloroform extracts. Add 0.1 g of *sodium bicarbonate* and shake for three minutes. Centrifuge the aqueous layer and transfer 10.0 ml to a round-bottomed flask with a ground-glass stopper. Add 20 ml of *ferric chloride solution* and mix. Heat in water-bath under a reflux condenser for twenty minutes, with frequent shaking, until the precipitate is dissolved. Cool, transfer to a separator and shake with three quantities, each of 25 ml, of *solvent ether*. Combine the ether extracts and wash with two quantities, each of 15 ml, of *water*. Transfer the ether extracts to a 100-ml volumetric flask and dilute to volume with *solvent ether*. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of *N potassium hydroxide*. Filter, if necessary, through a sintered glass filter. Measure the *extinction* immediately of a 1-cm layer of the resulting solution at 500 nm, Appendix 5.15 A. Calculate the content of hydroxyanthracene derivatives as sennoside B, taking 200 as the value of E(1 per cent, 1-cm) at 500 nm.

Storage : Store in containers protected against attack by insects and rodents.

Senna Leaf

Category : Cathartic.

Dose : 0.6 to 2.0 g.

Standards : Senna Leaf consists of the dried leaflets of *Cassia acutifolia* Delile known in commerce as Alexandrian Senna or of *Cassia angustifolia* Vahl known in commerce as Tinnevally Senna. It contains not less than 2.0 per cent of hydroxyanthracene derivatives, calculated as sennoside B.

Description : *Unground Alexandrian Senna – C. acutifolia* occurs as greyish-green, thin fragile leaflets, lanceolate, mucronate, asymmetrical at the base, 20 to 40 mm long and 5 to 15 mm wide, the maximum width being at a point slightly below the centre, lamina slightly undulant, both surfaces covered with fine short hairs. Pinnate venation with lateral veins leaving the midrib at an angle of about 60° and anastomosing to form a ridge near the margin.

Unground Tinnevally Senna – C. angustifolia occurs as yellowish-green leaflets, elongated and lanceolate, slightly asymmetrical at the base, 30 to 50 mm long and 7 to 20 mm wide at the centre. The two surfaces are smooth with a very small number of short hairs, and frequently marked with transverse or oblique lines.

Odour, slight and characteristic; taste, mucilaginous at first and then slightly bitter and unpleasant.

Examined under a microscope it shows pollygonal epidermal cells with straight walls and frequently containing mucilage; numerous, broadly elliptical stomata mostly from 20 to 35 μm in length, usually bordered by two neighbour-cells with their long axes parallel to that of the stoma, and rarely, though more frequently in Alexandrian Senna a third epidermal cell at the end of the stoma. The hairs are nonglandular, one-celled, conical, often curved, with thick papillose walls, from 100 to 350 μm in length. Palisade cells in a single layer underlie both surfaces except in the midrib region where they occur only beneath the upper epidermis. A meristele occurs in the midrib composed of several radially arranged fibrovascular bundles, the latter separated by narrow vascular rays and supported above and below by arcs of lignified pericyclic fibres. Calcium oxalate occurs in rosette aggregates in the spongy parenchyma and in six to eight-sided prisms in the crystal fibres, which lie on the outer surface of each group of pericyclic fibres.

The powder is light-green to greenish-yellow in colour and consists of polygonal epidermal cells showing stomata of the paracytic type. Unicellular hairs, conical in shape, with warted walls, isolated or attached to fragments of epidermis. Fragments of vascular bundles with a crystal sheath of prismatic crystals of calcium oxalate. Cluster crystals isolated or in fragments of parenchyma.

Identification : Complies with the test described under Senna Fruit.

Foreign organic matter : Not more than 1.0 per cent, Appendix 3.3.22.

Stalks : Not more than 2.0 per cent.

Sulphated ash : Not more than 12.0 per cent, Appendix 3.2.7.

Acid-insoluble ash : Not more than 2.0 per cent, Appendix 3.3.22.

Assay : Carry out the **Assay** described under Senna Fruit, using 0.25 g, accurately weighed.

Storage : Store in containers protected against attack by insects and rodents.

Shark Liver Oil

Category : Antixerophthalmic Vitamin.

Dose : 0.1 to 1.0 ml.

Description : Pale yellow to brownish-yellow oil; odour, fishy but not rancid; taste, bland or fishy.

Solubility : Slightly soluble in *alcohol*; miscible with *solvent ether*, with *chloroform*, and with *light-petroleum (boiling-range 60° to 80°)*.

Standards : Shark Liver Oil is the oil obtained from the fresh or carefully preserved livers of the shark and other allied species. It contains not less than 6000 Units of Vitamin A activity per gram.

Identification : A solution of one drop in 1 ml of *chloroform*, when shaken with one drop of *sulphuric acid*, acquires a light violet colour, changing to purple and finally to brown or blue.

Wt. per ml : Between 0.90 and 0.92g, determined at 20°, Appendix 5.19.

Refractive index : Between 1.459 and 1.477, determined at 40°, Appendix 5.14.

Acid value : Not more than 2.0, Appendix 3.3.15.

Saponification value : Not greater than 200, Appendix 3.3.20.

Unsaponifiable matter : Between 2.0 and 10.0 per cent, Appendix 3.3.21.

Iodine value : Not less than 90, Appendix 3.3.18.

Clarity of benzene solution : Transfer 5 ml to a centrifuge tube and add 5 ml of *benzene*; centrifuge for twenty-five minutes at 25°; no precipitate forms and a clear solution remains.

Assay : Determine the Vitamin A activity by the *assay of Vitamin A*, Appendix 3.3.24.4.

Storage : Store in well-filled, well-closed, light-resistant containers.

Dilute Shark Liver Oil

Category : Antixerophthalmic and antirachitic vitamins.

Dose : 4 to 12 ml daily, in divided doses.

Description : Pale yellow liquid; odour and taste, slightly fishy but not rancid.

Solubility : Slightly soluble in *alcohol*; miscible with *solvent ether*, with *chloroform*, and with *light petroleum (boiling-range 40° to 60°)*.

Standards : Dilute Shark Liver Oil is Shark Liver Oil diluted with decolorised and deodorised Arachis Oil and fortified with Vitamin D. It contains not less than 1000 Units of Vitamin A activity and not less than 100 Units of Vitamin D activity per gram.

Identification : Complies with the **Identification** test described under Shark Liver Oil.

Wt. per ml : Between 0.906 and 0.920 g, determined at 20°, Appendix 5.19.

Refractive index : Between 1.46 and 1.47, determined at 40°, Appendix 5.14.

Acid value : Not more than 1.0, Appendix 3.3.15.

Saponification value : Not more than 200, Appendix 3.3.20.

Unsaponifiable matter : Not more than 2.0 per cent, Appendix 3.3.21.

Iodine value : Between 90 to 130, Appendix 3.3.18.

Assay : (1) *For Vitamin A activity*—Carry out the *assay of Vitamin A*, Appendix 3.3.24.4, and express the result in Units of Vitamin A activity per gram.

(2) *For Vitamin D activity*—Carry out the *assay of Vitamin D*, Appendix 3.3.24.5, and express the result in Units of Vitamin D activity per gram.

Storage : Store in well-filled, well-closed, light-resistant containers.

Shark Liver Oil with Vitamin D

Category : Antixerophthalmic and antirachitic vitamins.

Dose : 0.5 to 1.5 ml daily, in divided doses.

Description : Pale yellow to brownish-yellow oil; odour, fishy but not rancid; taste, bland or fishy.

Solubility : Slightly soluble in *alcohol*; miscible with *solvent ether*, with *chloroform*, with *light petroleum (boiling-range 60° to 80°)*.

Standards : Shark Liver Oil with Vitamin D is Shark Liver Oil fortified with Vitamin D. It contains not less than 6000 Units of Vitamin A activity and not less than 1000 Units of Vitamin D activity per gram.

Acid value : Not more than 2.0, Appendix 3.3.15.

Assay : (1) *For Vitamin A activity*—Carry out the *assay of Vitamin A*, Appendix 3.3.24.4, and express the result in Units of Vitamin A activity per gram.

(2) *For Vitamin D activity*—Carry out the *assay of Vitamin D*, Appendix 3.3.24.5, and express the results in Units of Vitamin D per gram.

Storage : Store in well-filled, well-closed, light-resistant containers.

Shellac

Lac

Category : Pharmaceutical aid (tablet coating agent).

Description : Lemon-yellow to brownish-orange thin scale or hard, brittle masses; odourless or with faint odour; tasteless.

Solubility : Insoluble in *water*; soluble in warm *alcohol*; almost completely soluble in solutions of alkali hydroxides and of *borax*.

Standards : Shellac consists of a resinous substance prepared from a secretion that encrusts the bodies of a scale insect, *Laccifer lacca* Kerr (Fam. Ciccidae).

Acid value : Between 50 and 70, determined by the following method: Weigh accurately about 2 g and dissolve, with the aid of gentle heat, in 50 ml of *alcohol*, previously neutralised to *alcoholic thymol blue solution*. Titrate with *0.1N alcoholic potassium hydroxide* using *alcoholic thymol blue solution* as an external indicator.

Calculate the acid value from the formula: $\frac{a \times 5.61}{W}$

where a = number of ml of *0.1N alcoholic potassium hydroxide*.

W = weight in g of the sample.

Alcohol-insoluble matter : Not more than 2.0 per cent, determined by the following method: Weigh accurately about 5 g in an extraction thimble, cover with *alcohol* and allow to stand for sixteen hours. Place in an apparatus for the *continuous extraction of drugs*, extract with *alcohol* for four hours, dry the residue for three hours at 100° and weigh.

Colophony : Dissolve 2 g by shaking with 10 ml of *ethyl alcohol*, add slowly, with shaking 50 ml of *light petroleum (boiling range 40° to 60°)* to wash with two successive portions, each of 50 ml, of *water*, filter the washed alcohol-light petroleum solution, and evaporate to dryness, to the residue add 2 ml of a mixture of one volume of *liquified phenol* and two volumes of *carbon tetrachloride*, and transfer to a cavity of a colour-reaction porcelain tile; fill an adjacent cavity with a mixture of one volume of *bromine* and four volumes of *carbon tetrachloride*, and cover both cavities with an inverted watch-glass; no purple or deep indigo-blue colour is produced in the liquid containing the residue.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 1.0 per cent, Appendix 3.2.7.

Storage : Store in well-closed containers.

Silver Nitrate

$AgNO_3$ Mol. Wt. 169.87

Category : Local anti-infective.

Description : Colourless crystals or white crystalline powder; odourless; taste, bitter and metallic.

Solubility : Very soluble in *water*; sparingly soluble in *alcohol*; slightly soluble in *solvent ether*.

Standards : Silver Nitrate contains not less than 99.5 per cent and not more than the equivalent of 100.5 per cent of $AgNO_3$.

Identification : A solution (1 in 50) gives the reactions of *silver*, and of *nitrates*, Appendix 3.1.

pH : Between 5.4 and 6.4 determined in a 4.0 per cent w/v solution, Appendix 5.10.

Clarity and colour of solution : A solution of 2 g in 20 ml of *water* is clear and colourless.

Bismuth, copper and lead : To a solution of 1 g in 5 ml of *water*, add a slight excess of *dilute ammonia solution*; the mixture remains clear and colourless.

Foreign substances : To 30 ml of a 4.0 per cent w/v solution add 7.5 ml of *2N hydrochloric acid*, shake vigorously, filter and evaporate 10 ml of the filtrate to dryness on a water-bath; the residue weighs not more than 1 mg.

Assay : Weigh accurately about 0.5 g and dissolve in 50 ml of *water*, add 2 ml of *nitric acid*, and titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N ammonium thiocyanate* is equivalent to 0.01699 g of $AgNO_3$.

Storage : Store in tightly-closed, light-resistant containers.

Smallpox Vaccine (Freeze-Dried)

Category : Immunising agent.

Dose : For prophylaxis of smallpox, about 0.02 ml applied to the skin and inoculated by scarification or pressure.

Standards : Smallpox Vaccine (freeze-dried) is a freeze-dried suspension of the living virus of vaccinia, of a strain capable of protecting man against smallpox.

The virus is grown in the skin of healthy animals, usually young cattle or sheep. The product is suspended in a solution having stabilising properties and then distributed into its final sterile containers and freeze-dried. It may contain a suitable preservative.

Description : Almost white powder which reconstitutes to yield a viscid, straw-coloured liquid.

When reconstituted as stated on the label, the vaccine complies with the following requirements.

Identification : Inoculate the vaccine into the scarified skin of a rabbit or into cell culture. It induces the formation of characteristic lesions. After neutralisation of the virus by a specific anti-serum of high potency, the preparation does not induce the formation of lesions.

Living contaminants : Carry out the *test for living contaminants in smallpox vaccine*, Appendix 2.25. The vaccine is free from such contaminants. The total number of non-pathogenic bacteria does not exceed 500 per ml.

Sterility : The requirement for Sterility does not apply to the vaccine.

Undue toxicity : Complies with the test for *undue toxicity for vaccines and sera*, Appendix 2.37 injecting each mouse with 0.2 ml and each guinea-pig with 0.5 ml.

Potency : Carry out the *determination of potency of smallpox vaccine*, Appendix 2.26.

Stability : Keep the freeze-dried vaccine at a temperature between 35° and 37° for not less than four weeks, dissolve in the stated quantity of the liquid stated on the label and repeat the test for potency; the potency is not less than one-tenth of that originally found.

Storage : Store at a temperature between 2° and 8°. The reconstituted vaccine may be expected to retain its potency for seven days when kept at a temperature between 2° and 8°.

Labelling : The label on the container states (1) the directions for reconstituting the vaccine; (2) that the vaccine is to be administered into the skin by scarification or other suitable means and is not to be injected; (3) the animal in which the vaccine was prepared; (4) the date of expiry which should not exceed six months from the date on which the test for potency was carried out.

Snake Venom Antiserum

Antivenin, Antivenom Serum, Venom Antitoxin

Category : Passive immunising agent.

Dose : Usually 20 to 50 ml, injected intravenously and into and around the bitten site as soon as possible, followed by further amounts, as necessary.

Description : *Native antiserum preparation* – Yellow, yellowish-brown or greenish-yellow, clear to slightly opalescent liquid.

Freeze-dried serum – Cream-coloured powder.

Standards : Snake Venom Antiserum is a sterile preparation containing specific antitoxic globulins or their derivatives, that have the power of neutralising the venom of one or more kinds of snake. It may be obtained from the serum of healthy animals such as horses, mules, sheep or bulls, immunised against venoms of specific kinds of snakes, usually vipers and cobras. It may be a native serum, or a preparation from native serum or a freeze-dried product. It may contain a suitable preservative.

Identification : It renders the corresponding venom or venoms, harmless to susceptible animals.

pH : *Native antiserum* – Between 7.0 and 8.5, Appendix 5.10.

Other preparations – Between 6.0 and 7.0, Appendix 5.10.

Other requirements : Complies with the requirements for **General tests** stated under Antisera.

Potency : In view of the numerous toxic fractions and venoms and the varying requirements of different localities, no standard for potency is indicated.

The potency of snake venom antiserum should be such that the dose stated on the label will completely neutralise the maximum amount of venom likely to be delivered by a bite by a snake. This is determined either by estimating the ability of the antiserum being examined to protect mice or other suitable animals, against the lethal effect of a fixed dose of a standard snake venom of the relevant species, or by comparing its ability to do so with that of a standard preparation of antiserum of established potency. Assays should be done against the standard preparation at two or more dose-levels.

Storage : Store in single-dose containers and avoid exposure to excessive heat.

Labelling : The label on the container states (1) the nominal volume of contents and in the case of a freeze-dried preparation, the directions for reconstitution; (2) the species of snake against whose venom the antiserum is effective; (3) the name of the animal serum from which the antiserum has been prepared; (4) the name and proportion of any added preservative; (5) the storage conditions; (6) the date after which the preparation is not intended to be used.

Sodium Acetate

$CH_3COONa\ ,3H_2O$

$C_2H_3NaO_2,3H_2O$ Mol. Wt. 136.08

Category : Pharmaceutical aid (for peritoneal dialysis fluids).

Description : Colourless crystals or white, granular crystalline powder; odourless or with a slight odour of acetic acid; taste, slightly saline and bitter.

Solubility : Very soluble in *water*; soluble in *alcohol*.

Standards : Sodium Acetate contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_2H_3NaO_2,3H_2O$.

Identification : It gives the reactions of *sodium* and of *acetates*, Appendix 3.1.

Clarity and colour of solution : A 10.0 per cent w/v solution is clear and colourless.

pH : Between 7.5 and 9.0, determined in a 5 per cent w/v solution, Appendix 5.10.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Calcium and magnesium : Mix 200 ml of *water* with 10 ml of *ammonia buffer solution*, 0.1 g of *mordant black 11 mixture* and 2 ml of *0.05M zinc chloride;* add dropwise, *0.05M disodium ethylenediaminetetraacetate* until the colour changes from violet-red to green. To this solution add 10 g of the substance being examined, shake to dissolve, and titrate with *0.05M disodium ethylenediaminetetraacetate* until the green colour is restored; not more than 0.25 ml of *0.05M disodium ethylenediaminetetraacetate is required*.

Heavy metals : Not more than 10 parts per million, determined by Method A on 2.0 g dissolved in sufficient *water* to produce 20 ml, Appendix 3.2.4.

Iron : 4.0 g complies with the *limit test for iron*, Appendix 3.2.5.

Chloride : 1.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 1.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Reducing substances : Dissolve 1.0 g in 100 ml of boiling *water*, add 5 ml of *dilute sulphuric acid* and 0.5 ml of *0.01N potassium permanganate*, and boil gently for five minutes; the pink colour is not entirely discharged.

Assay : Weigh accurately about 0.25 g, dissolve in 50 ml of *glacial acetic acid*, add 5 ml of *acetic anhydride* and allow to stand for thirty minutes. Add 50 ml of *glacial acetic acid* and titrate with *0.1N perchloric acid*, using 0.3 ml of *1-naphtholbenzein solution* as indicator.

Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.01361 g of $C_2H_3NaO_2,3H_2O$.

Storage : Store in tightly-closed containers.

Sodium Acid Phosphate

Sodium Dihydrogen Phosphate

$NaH_2PO_4,2H_2O$ Mol. Wt. 156.01

Category : Urinary acidifier.

Dose : 2 to 4 g.

Description : Colourless crystals or white crystalline powder; odourless; taste, acid and saline. Slightly deliquescent.

Solubility : Freely soluble in *water*; practically insoluble in *alcohol*.

Standards : Sodium Acid Phosphate contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of $NaH_2PO_4, 2H_2O$.

Identification : It gives the reactions of *sodium*, and of *phosphates*, Appendix 3.1.

pH : Between 4.3 and 4.5, determined in a 5.0 per cent w/v solution, Appendix 5.10.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Calcium and magnesium : Dissolve 2 g in 50 ml of *water*, add 3 ml of *dilute ammonia solution* and set aside for five minutes; no turbidity is produced.

Heavy metals : Not more than 20 parts per million determined by Method A, Appendix 3.2.4, on a solution prepared by dissolving 1.0 g in 1 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml.

Chloride : 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

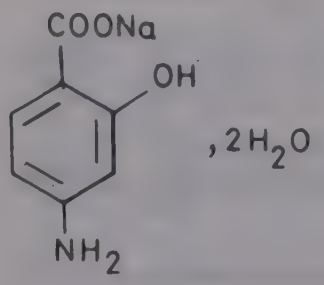
Disodium phosphate : Dissolve 1 g in 50 ml of *water*, and titrate with *0.1N sulphuric acid*, using *bromocresol green solution* as indicator; not more than 1 ml of *0.1N sulphuric acid* is required.

Assay : Weigh accurately about 3 g and dissolve in 100 ml of *water*, add 25 g of *sodium chloride*, and titrate with *0.5N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.5N sodium hydroxide* is equivalent to 0.0780 g of $NaH_2PO_4,2H_2O$.

Storage : Store in well-closed containers.

Sodium Aminosalicylate

Sodium PAS



$C_7H_6NNaO_3, 2H_2O$ Mol. Wt. 211.15

Category : Anti bacterial (Tuberculostatic).

Dose : 10 to 15 g daily, in divided doses.

Description : White to cream-coloured, crystalline powder; odourless; taste, saline and bitter with a sweet after-taste.

Solubility : Freely soluble in *water*; sparingly soluble in *alcohol*; very slightly soluble in *chloroform* and in *solvent ether*.

Standards : Sodium Aminosalicylate is the dihydrate of sodium 4-amino-2-hydroxy-benzoate. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_7H_6NNaO_3$ calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** tests (A) and (B), described under Calcium Aminosalicylate.

(B) A solution (1 in 20) gives the reactions of *sodium*, Appendix 3.1.

Clarity and colour of solution : A 10.0 per cent w/v solution is clear and has not more than a faint yellow colour.

pH : Between 6.5 and 8.5, determined in a 2.0 per cent w/v solution, Appendix 5.10.

Arsenic, chloride, sulphate : Complies with the requirements stated under Calcium Aminosalicylate.

3-Aminophenol : Not more than 0.2 per cent, determined by the following method: Weigh accurately about 0.69 g and carry out the test for **3-Aminophenol** described under Calcium Aminosalicylate, beginning at the words "add 1.8 ml of *N sodium hydroxide* . . .". Calculate the percentage of 3-aminophenol in the sample from the formula:

$$\frac{(A-0.32)}{1.16}$$

5-Aminosalicylic acid : Dissolve 0.2 g in 2 ml of *water* and add 2 ml of *2N sodium hydroxide*, a few crystals of *resorcinol* and 0.15 ml of *iodine solution*; a yellow, but not violet, colour is produced.

Hydrogen sulphide and sulphur dioxide : Dissolve 0.5 g in 5 ml of *N sodium hydroxide*. add 6 ml of *3N*

hydrochloric acid and stir vigorously; no odour of hydrogen sulphide or sulphur dioxide is perceptible. A piece of moistened *lead acetate paper* held over the mixture does not become discoloured.

Heavy metals : Not more than 30 parts per million, determined on 0.66 g by Method B, Appendix 3.2.4.

Loss on drying : Between 16.0 per cent and 17.5 per cent, determined on 0.5 g by drying in an oven at 105° Appendix 5.8.

Assay : Carry out the **Assay** described under Calcium Aminosalicylate. Each ml of *0.1M sodium nitrite* is equivalent to 0.01752 g of $C_7H_6NNaO_3$.

Storage : Store in well-closed, light-resistant containers.

Sodium Aminosalicylate Granules

Sodium PAS Granules

Category : Antibacterial (Tuberculostatic).

Dose : Sodium Aminosalicylate, 10 to 15 g daily, in divided doses.

Standards : Sodium Aminosalicylate Granules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sodium Aminosalicylate, $C_7H_6NNaO_3$, $2H_2O$, the stated amount being not less than 0.8 g of Sodium Aminosalicylate in 1 g. The granules may be coated and coloured.

Identification : (A) Extract a quantity of the powdered granules equivalent to about 3 g of Sodium Aminosalicylate in 40 ml of *water* and filter. To the filtrate add 15 ml of *dilute acetic acid* and set aside until precipitation occurs. Collect the precipitate on a filter, wash with *water*; the residue after drying at 105° for thirty minutes complies with **Identification** test (A) described under Calcium Aminosalicylate.

(B) The residue obtained in **Identification** test (A) melts at about 145°, Appendix 5.11.

Disintegration : Carry out the *disintegration test for tablets*, Appendix 5.6.1, on 2 g, using the tube fitted at both ends with the discs of rust-proof wire gauze complying with the requirements for a No. 36 *sieve*, Appendix 1.2. Maximum time, thirty minutes.

3-Aminophenol : Not more than 0.75 per cent when determined by the following method: Weigh accurately a quantity of the powdered granules equivalent to 1.38 g of Sodium Aminosalicylate and complete the test for **3-Aminophenol** described under Calcium Aminosalicylate Tablets, beginning at the words "transfer to a 100-ml graduated flask...". Calculate the percentage of 3-aminophenol in the sample from the formula:

$$\frac{(A-0.32)}{1.16}$$

Assay : Finely powder the granules. Weigh accurately a quantity of the powder equivalent to about 0.6 g of Sodium Aminosalicylate and dissolve in 75 ml of *water* with occasional shaking. Make upto 100.0 ml with *water*. Filter if necessary. To 5.0 ml of the filtrate add 10 ml of *hydrochloric acid*, 1 g of *potassium bromide* and cool to 15°; carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02112 g of $C_7H_6NNaO_3$, $2H_2O$.

Storage : Store in tightly-closed, light-resistant containers.

Sodium Aminosalicylate Tablets

Sodium PAS Tablets

Category : Antibacterial (Tuberculostatic).

Dose : Sodium Aminosalicylate, 10 to 15 g daily, in divided doses.

Usual strengths : 0.5 g; 1 g.

Standards : Sodium Aminosalicylate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sodium Aminosalicylate, $C_7H_6NNaO_3$,$2H_2O$. The tablets may be coated.

Identification : (A) Comply with **Identification** test (A) described under Calcium Aminosalicylate Tablets.

(B) The powdered tablets give the reactions of *sodium*, Appendix 3.1.

Other requirements : Comply with the requirements stated under Tablets.

3-Aminophenol : Not more than 0.75 per cent, determined by the following method: Weigh and powder 20 tablets, weigh accurately a quantity of the powder equivalent to 1.38 g of sodium aminosalicylate and complete the test as described under Calcium Aminosalicylate Tablets beginning at the words "transfer to a 100-ml graduated flask....". Calculate the percentage of 3-aminophenol in the samples from the formula:

$$\frac{(A-0.32)}{1.16}$$

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.6 g of Sodium Aminosalicylate, add 75 ml of *water*. Shake until

solution of the sodium aminosalicylate is complete and dilute to 100 ml. Filter, if necessary, rejecting the first 20 ml of the filtrate. To 50 ml of the filtrate add 10 ml of *hydrochloric acid*, 1 g of *potassium bromide* and cool to 15°. Carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02112 g of $C_7H_6NNaO_3, 2H_2O$.

Storage : Store in tightly-closed, light-resistant containers.

Sodium Antimony Gluconate

Sodium Stibogluconate

Category : Systemic leishmaniacidal.

Dose : By intramuscular or intravenous injection, 0.6 to 2.0 g daily, for ten to thirty days.

Description : White, amorphous powder; practically odourless.

Solubility : Very soluble in *water*; practically insoluble in *alcohol* and in *solvent ether*.

Standards : Sodium Antimony Gluconate is a pentavalent antimony compound. It contains not less than 30.0 per cent and not more than 34.0 per cent of total antimony, calculated with reference to the dried substance.

Identification : (A) When heated, it chars without melting, emitting an odour of burnt sugar; the residue gives the reactions of *antimony*, and of *sodium*, Appendix 3.1.

(B) A 10 per cent w/v solution is *dextro*-rotatory.

Stability of solution : Heat a solution containing the equivalent of 10 per cent w/v of total antimony in an autoclave at 115° for thirty minutes; the resulting solution is almost colourless.

pH : Between 5.0 and 6.0, determined on the solution obtained in the *test for stability of solution*, Appendix 5.10.

Chloride : To 5 ml of a 5.0 per cent w/v solution add one drop of *dilute nitric acid* and one drop of *silver nitrate solution*; no immediate precipitate is produced.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Dissolve 0.6 g in 2 ml of *water* and add 1 ml of *sodium sulphide solution*; the solution remains clear.

Calcium : Dissolve 0.6 g in 2 ml of *water*, add 1 ml of *dilute ammonia solution* and 0.5 ml of 2.5 per cent w/v solution of *ammonium oxalate*; the solution remains clear.

Trivalent antimony : Dissolve 0.15 g in 1 ml of *water* and acidify with 1 ml of *dilute acetic acid*. Add one drop of *0.01N iodine* and then a drop of *starch solution*; a blue colour develops.

Loss on drying : Between 10.0 per cent and 15.0 per cent, determined on 1.0 g by drying "in vacuo at 130°", Appendix 5.8.

Assay : Transfer 0.25 g to a round-bottomed, 300-ml boiling-flask of hard glass having a medium-length neck, add 10 ml of *nitric acid* and 5 ml of *sulphuric acid*, and heat cautiously over a small flame, keeping the liquid in motion by rotating the flask; remove the flask from the flame at the onset of the first vigorous reaction until this subsides and then continue heating until white fumes are evolved. Allow the liquid to cool, add 1 ml of *nitric acid*, and again heat until white fumes are evolved. Add 1 g of *ammonium sulphate*, again heat to the point of fuming, cool thoroughly, and add 1 g of *tartaric acid* and 60 ml of *water*. To the clear, almost colourless solution add 1 g of *potassium iodide* and boil the solution gently for about five minutes or until free iodine is expelled, when the liquid should have a pure yellow colour. Cool, make the solution just alkaline with a 50 per cent w/v solution of *sodium hydroxide* (approximately 15 ml is usually required), cool, make just acid with *dilute sulphuric acid*, add an excess of *sodium bicarbonate*, and titrate with *0.05N iodine* adding *starch solution* if necessary. Each ml of *0.05N iodine* is equivalent to 0.003044 g of total antimony.

Storage : Store in well-closed containers.

Sodium Antimony Gluconate Injection

Sodium Stibogluconate Injection

Category : Systemic leishmaniacidal.

Dose : Sodium Antimony Gluconate, by intramuscular or intravenous injection, 0.6 to 2.0 g daily, for ten to thirty days.

Usual strength : The equivalent of 0.1 g of total antimony per ml.

Description : Clear, colourless or faintly straw-coloured liquid.

Standards : Sodium Antimony Gluconate Injection is a sterile solution of Sodium Antimony Gluconate in Water for Injection. It contains not less than 9.5 per cent w/v and not more than 10.5 per cent w/v of total antimony.

Identification : (A) It is *dextro*-rotatory.

(B) Dilute 2 ml to 10 ml with *water* and pass *hydrogen sulphide* through the solution for several minutes; an orange precipitate is produced.

(C) The residue after evaporation to dryness and incineration gives the reactions of *antimony*, and of *sodium*, Appendix 3.1.

pH : Between 4.5 and 6.0, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

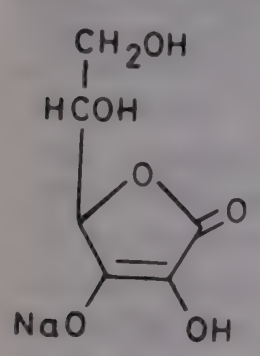
Assay : Carry out the **Assay** described under Sodium Antimony Gluconate, using 1 ml.

Storage : Store in single-dose or multiple-dose, light-resistant containers.

Labelling : The strength is stated as the equivalent amount of total antimony per ml.

Sodium Ascorbate

Monosodium L-ascorbate



$C_6H_7NaO_6$ Mol. Wt. 198.11

Category : Vitamin C.

Dose : The equivalent of up to 1 g of ascorbic acid daily.

Description : White or very faintly yellow crystals or crystalline powder; odourless or nearly odourless; darkens gradually on exposure to light

Solubility : Freely soluble in *water*; very slightly soluble in *alcohol*; insoluble in *chloroform* and in *solvent ether*.

Standards : Sodium Ascorbate is the monosodium salt of L-ascorbic acid. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_6H_7NaO_6$, calculated with reference to the dried substance.

Identification : (A) Add 1 ml of *0.1N hydrochloric acid* to 4 ml of a 2 .0 per cent w/v solution; the resulting solution complies with **Identification** tests (A) and (B) described under Ascorbic Acid.

(B) A 2.0 per cent w/v solution gives the reactions of *sodium*, Appendix 3.1.

pH : Between 7.0 and 8.0, determined in a 10.0 per cent w/v solution, Appendix 5.10.

Specific optical rotation : Between +103° and +108°, calculated with reference to the dried substance and determined in a 10.0 per cent w/v solution, Appendix 5.12.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Loss on drying : Not more than 0.25 per cent, determined on 1.0 g by drying "in vacuo" for 24 hours, Appendix 5.8.

Assay : Weigh accurately about 0.4 g and dissolve in a mixture of 100 ml of *carbon dioxide-free water* and 25 ml of *dilute sulphuric acid*. Titrate immediately with *0.1N iodine*, adding *starch solution* towards the end of the titration, as indicator. Each ml of *0.1N iodine* is equivalent to 0.009905 g of $C_6H_7NaO_6$.

Storage : Store in tightly-closed, light-resistant containers.

Sodium Aurothiomalate

Category : Antirheumatic.

Dose : By intramuscular injection, 10 mg, increasing gradually to 100 mg weekly, to a total of 750 mg, then in decreasing amounts.

Description : Fine, pale yellow powder; odour slight. Hygroscopic.

Solubility : Very soluble in *water*; insoluble in *alcohol* and in *solvent ether*.

Standards : Sodium Aurothiomalate consists mainly of the disodium salt of aurothiosuccinic acid. It contains not less than 44.5 per cent and not more than 46.0 per cent of Au, and not less than 10.8 per cent and not more than 11.5 per cent of Na; both calculated with reference to the dried substance.

Identification : (A) Ignite 0.1 g and extract the residue with 10 ml of *dilute hydrochloric acid*. The solution, after neutralisation, if necessary, gives the reactions of *sodium*, and of *sulphates*, Appendix 3.1.

(B) Dissolve the residue obtained in **Identification** test (A) by warming with 2 ml of a mixture of 3 volumes of *hydrochloric acid* and 1 volume of *nitric acid* and dilute to 20 ml with *water*. The solution complies with the following tests: To 0.2 ml and 20 ml of *water*, boil, pour

the boiling solution into 5 ml of *stannous chloride solution* and mix; a purple colour is produced. To 2 ml add 2 ml of *hydrogen peroxide solution* and 1 ml of *sodium hydroxide solution*; a precipitate is produced which appears brownish-black by reflected light and bluish-green by transmitted light.

pH : Between 6.0 and 7.0 determined in a 10 per cent w/v solution, Appendix 5.10.

Loss on drying : Not more than 2.0 per cent determined by drying 1 g "in vacuo" for twenty-four hours, Appendix 5.8.

Assay : (a) *For Au*—Weigh accurately about 0.2 g, heat with 10 ml of *sulphuric acid* and continue to boil gently until a clear pale yellow liquid is produced. Cool, add about 1 ml of *nitric acid*, drop by drop, and boil again for one hour. Cool, dilute with 70 ml of *water*, boil for five minutes, filter, wash the residue of gold, Au, with hot *water*, dry, and ignite for three hours at a temperature not lower than 600°.

(b) *For Na*—Evaporate to dryness the filtrate and washings obtained in the *Assay for Au*, moisten with *sulphuric acid*, and ignite for three hours at 600° Each g of residue is equivalent to 0.03237 g of Na.

Storage : Store in tightly-closed, light-resistant containers.

Sodium Aurothiomalate Injection

Category : Antirheumatic.

Dose : Sodium Aurothiomalate. By intramuscular injection, 10 mg, increasing gradually to 100 mg, weekly, to a total of 750 mg,. then decreasing amounts.

The total dose in one course of treatment should not exceed 1.0 g.

Usual strengths : 10 mg in 1 ml; 20 mg in 1 ml.

Standards : Sodium Aurothiomalate Injection is a sterile solution of Sodium Aurothiomalate in Water for Injection. The content of gold, Au, is not less than 42.3 per cent and not more than 48.3 per cent of the stated amount of Sodium Aurothiomalate.

Description : Pale-yellow solution.

Identification : (A) To a volume equivalent to 20 mg of Sodium Aurothiomalate, add 2 ml of *strong hydrogen peroxide solution* and 1 ml of *sodium hydroxide solution* and boil for half a minute. A colloidal precipitate is produced which appears bluish-green by transmitted light.

(B) To a volume equivalent to 0.1 g of Sodium Aurothiomalate, add 1 ml of *potassium cyanide solution* and 1 ml of *sodium nitroprusside solution*, a deep magenta colour is obtained.

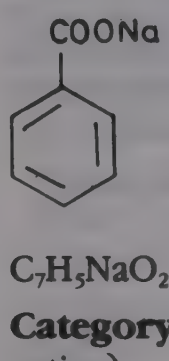
Colour of solution : Dilute, if necessary, with *water* to give a solution containing 1 per cent w/v of Sodium Aurothiomalate; the colour of the solution is not deeper than that of a 0.005 per cent w/v solution of *potassium dichromate*.

Other requirements : Complies with the requirements stated under Injections.

Assay : To an accurately measured volume equivalent to about 0.1 g of Sodium Aurothiomalate, add 0.4 g of *potassium bromide* and 5 ml of *nitric acid*. Slowly evaporate to dryness and continue heating until fumes cease to be evolved. Allow to cool, add 50 ml of *water*, warm, filter, wash the filter with hot *water*, dry and ignite the residue of gold, Au, for three hours at a temperature not lower than 600°.

Storage : Store in light-resistant containers.

Sodium Benzoate



$C_7H_5NaO_2$ Mol. Wt. 144.11

Category : Pharmaceutical aid (antifungal preservative).

Description : White, amorphous, granular or crystalline powder; odourless or with a faint odour; taste, unpleasant, sweetish and saline.

Solubility : Freely soluble in *water*; sparingly soluble in *alcohol*.

Standards : Sodium Benzoate contains not less than 99.0 per cent of $C_7H_5NaO_2$, calculated with reference to the dried substance.

Identification : (A) Fuses on heating, emitting vapours having a characteristic odour, then chars and finally leaves a residue of sodium carbonate and carbon.

(B) To a 10 per cent w/v solution add *ferric chloride test-solution*, a buff-coloured precipitate is formed; add *dilute hydrochloric acid*, a white crystalline precipitate is produced.

(C) A solution (1 in 20) gives the reactions of *sodium*, and of *benzoates*, Appendix 3.1.

Acidity or Alkalinity : Dissolve 2 g in 20 ml of hot, freshly boiled *water*, and titrate with *0.1N sodium*

hydroxide or *0.1N sulphuric acid*, using *phenolphthalein solution* as indicator; not more than 0.5 ml of *0.1N sodium hydroxide* or *0.1N sulphuric acid* is required.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined by Method A, on 25 ml of a solution prepared by dissolving 2.0 g in 45 ml of *water* and 5 ml of *hydrochloric acid* and filtering, Appendix 3.2.4.

Chloride : Dissolve 1 g in 40 ml of *water*, add 1 ml of *nitric acid* and filter; the filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : Dissolve 0.5 g in 40 ml of *water*, add 1 ml of *hydrochloric acid* and filter; the filtrate complies with the *limit test for sulphates*, Appendix 3.2.8.

Chlorinated compounds : Complies with the test for **Chlorinated compounds** described under Benzoic Acid.

Loss on drying : Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.6 g, add 100 ml of *glacial acetic acid* and stir until complete solution is effected. Titrate with *0.1N perchloric acid* using *crystal-violet solution* as indicator. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.01441 g of $C_7H_5NaO_2$.

Storage : Store in well-closed containers.

Sodium Bicarbonate

$NaHCO_3$ Mol. Wt. 84.01

Category : Electrolyte replenisher; systemic alkaliser.

Dose : 1 to 4 g.

Description : White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline.

Solubility : Freely soluble in *water*; practically insoluble in *alcohol*.

Standards : Sodium Bicarbonate contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of $NaHCO_3$, calculated with reference to the dried substance.

Identification : It gives the reactions of *sodium*, and of *bicarbonates*, Appendix 3.1.

Clarity and colour of solution : A 5.0 per cent w/v solution is clear and colourless.

Carbonate : pH of a freshly prepared 5.0 per cent w/v solution in *carbon dioxide-free water*, not more than 8.6, Appendix 5.10.

Aluminium, calcium and insoluble matter : Boil 10 g with 50 ml of *water* and 20 ml of *dilute ammonia solution*, filter, and wash the residue with *water*; the residue, after ignition to constant weight, weighs not more than 1 mg.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Iron : Dissolve 2.5 g in 20 ml of *water* and 4 ml of *iron-free hydrochloric acid*, and dilute to 40 ml with *water*; the solution complies with the *limit test for iron*, Appendix 3.2.5.

Heavy metals : Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner:

Mix 4.0 g with 5 ml of *water* and 10 ml of *dilute hydrochloric acid*, heat to boiling, and maintain that temperature for one minute. Add one drop of *phenolphthalein solution* and sufficient *ammonia solution* dropwise to give the solution a faint pink colour. Cool and dilute to 25 ml with *water*, Appendix 3.2.4.

Chlorides : Dissolve 1.0 g in *water* with the addition of 2 ml of *nitric acid*; the solution complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphates : Dissolve 2 g in *water* with the addition of 2 ml of *hydrochloric acid*; the solution complies with the *limit test for sulphates*, Appendix 3.2.8.

Ammonium compounds : 1 g warmed with 10 ml of *sodium hydroxide solution* does not evolve ammonia.

Loss on drying : Not more than 0.25 per cent, determined on 4.0 g by drying over *silica gel* for four hours, Appendix 5.8.

Assay : Weigh accurately about 1 g, dissolve in 20 ml of *water*, and titrate with *0.5N sulphuric acid*, using *methyl orange solution* as indicator. Each ml of *0.5N sulphuric acid* is equivalent to 0.042 g of $NaHCO_3$.

Storage : Store in well-closed containers.

Sodium Carboxymethylcellulose

Sodium Cellulose Glycollate

Category : Pharmaceutical aid (suspending agent; tablet excipient; viscosity-increasing agent).

Description : White to cream-coloured powder or granules; odourless or almost odourless; hygroscopic.

Solubility : Soluble in *water*, insoluble in *alcohol*, in *solvent ether* and in most organic solvents.

Standards : Sodium Carboxymethylcellulose is the sodium salt of a polycarboxymethyl ether of cellulose. It contains not less than 6.5 per cent and not more than 9.5 per cent of sodium, Na, calculated with reference to the dried substance.

Identification : Disperse about 2 g in 150 ml of *water* and stir for thirty minutes continuously with the aid of a mechanical stirrer, until a clear solution is obtained. Dilute to 200 ml with *water*. Carry out the following tests on the resulting solution : (1) To 10 ml add sufficient *dilute hydrochloric acid* to give a pH of 0.5; a fine white precipitate is formed. (2) Boil 10 ml for five minutes; no precipitate is formed. (3) To 2 ml in a test-tube add five drops of *α-naphthol solution*; add carefully down the side of the tube 2 ml of *sulphuric acid* so that it forms a lower layer; a red purple colour develops at the interface. (4) To 5 ml add 5 ml of *barium chloride solution*; a fine white precipitate is produced. (5) It gives the reactions of *sodium*, Appendix 3.1.

pH : Between 6.0 and 8.0, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*, Appendix 5.10.

Viscosity : Not less than 80.0 per cent and not more than 120.0 per cent of that stated on the label for viscosity types of 100 cps. or less; and not less than 75.0 per cent and not more than 140.0 per cent of that stated on the label for viscosity types higher than 100 cps., determined in a 1.0 per cent w/v solution by the following method: Weigh accurately about 1.0 g calculated with reference to the dried substance, and stir for ten minutes with 100 ml of *water* heated to 85° to 90°, until the powder is completely wetted. Cool to room temperature and continue the stirring until the solution is of uniform consistency. Adjust the temperature to 25° ± 0.2° and determine the viscosity in a *suspended level viscometer*, Appendix 5.18, or in any other suitable viscometer.

Arsenic : Not more than 1 part per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined on 2 g by Method A, Appendix 3.2.4, using a test solution prepared in the following manner: To 2 g in a porcelain crucible add five drops of *sulphuric acid*, allowing each drop to run down the sides of the crucible. Cover the crucible, and heat gently on a hot plate until the volatile matter is removed. Ignite to 500° to 600° until the carbon is burned off. Cool, add 2 ml of *hydrochloric acid* and slowly evaporate on a water-bath to dryness. Moisten the residue with one drop of *hydrochloric acid*, add 10 ml of hot *water* and 0.5 ml of a 20 per cent w/v solution of *hydroxylamine hydrochloride* and digest for two minutes. Filter, wash with a few ml of *water*, and dilute with *water* to 25 ml.

Loss on drying : Not more than 10.0 per cent, determined on 0.5 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and disperse in 80 ml of *glacial acetic acid*. Heat on a water-bath for two hours. Cool and titrate with *0.1N perchloric acid*, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.002299 g of Na.

Storage : Store in well-closed containers.

Labelling : The label on the container states the viscosity of a 1.0 per cent w/v solution.

Sodium Chloride

NaCl Mol. Wt. 58.44

Category : Pharmaceutical aid (tonicity agent).

Description : Colourless crystals or white crystalline powder; odourless; taste, saline.

Solubility : Freely soluble in *water*, and slightly more soluble in boiling *water*; soluble in *glycerin*; slightly soluble in *alcohol*.

Standards : Sodium Chloride contains not less than 99.5 per cent and not more than the equivalent of 100.5 per cent of NaCl, calculated with reference to the dried substance. It contains no added substances.

Identification : It gives the reactions of *sodium*, and of *chlorides*, Appendix 3.1.

Acidity or Alkalinity : 5 g dissolved in 50 ml of *carbon dioxide-free water*, requires not more than 0.5 ml of *0.01N sodium hydroxide* or *0.01N hydrochloric acid* for neutralisation to *phenol red solution*.

Arsenic : Not more than 1 part per million, Appendix 3.2.1.

Iron : 1 g complies with the *limit test for iron*, Appendix 3.2.5.

Heavy metals : Not more than 5 parts per million, determined by Method A on a solution of 4.0 g in 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, Appendix 3.2.4.

Calcium and magnesium : Not more than 0.005 per cent, calculated as Ca and determined by the following method: Dissolve 20 g in 200 ml of *water*, and add 0.1 ml of *hydrochloric acid*, 5 ml of *strong ammonia-ammonium chloride solution*, five drops of *eriochrome black T solution* and titrate with *0.005M disodium ethylenediaminetetraacetate* to a blue end-point. Each ml of *0.005M*

disodium ethylenediamine tetraacetate is equivalent to 0.0002004 g of Ca.

Barium : Dissolve 2 g in 10 ml of *water*, and add 2 ml of *dilute sulphuric acid*; no turbidity is produced within two hours.

Sulphate : 2 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Iodide or Bromide : Finely powder 2 g and digest for three hours with 25 ml of warm *alcohol*, cool and filter. Evaporate the filtrate to dryness, dissolve the residue in 5 ml of *water*; add 1 ml of *chloroform* and cautiously add dropwise, with constant agitation, 5 drops of *dilute chlorine solution*; the chloroform does not acquire a violet, yellow or orange colour.

Sodium ferrocyanide : Dissolve 25 g in 80 ml of *water* in a glass-stoppered cylinder, add 2 ml of *ferrous sulphate solution*, 1 ml of *dilute sulphuric acid*, dilute with *water* to 100 ml and mix. The solution is not more intensely coloured than a mixture of 2 ml of *ferrous sulphate solution*, 1 ml of *dilute sulphuric acid* and sufficient *water* to produce 100 ml.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°, Appendix 5.8.

Assay : Weigh accurately about 0.1 g and dissolve in 50 ml of *water* in a glass-stoppered flask. Add 50.0 ml of *0.1N silver nitrate*, 3 ml of *nitric acid*, 5 ml of *nitrobenzene*, 2 ml of *ferric ammonium sulphate solution* and shake well. Titrate with *0.1N ammonium thiocyanate* until the colour becomes reddish-yellow. Each ml of *0.1N silver nitrate* is equivalent to 0.005844 g of NaCl.

Sodium Chloride intended for parenteral administration complies with the following additional requirement:

Potassium : Not more than 0.1 per cent, determined by *flame photometry*, Appendix 5.16 A, using a 1.0 per cent w/v solution and measuring at 768 nm. Use suitable dilutions in *water* of *potassium solution FP* for the standard solutions.

Storage : Store in well-closed containers.

Sodium Chloride Injection

Sodium Chloride Intravenous Infusion

Category : Fluid and Electrolyte replenisher; isotonic vehicle.

Standards : Sodium Chloride Injection is a sterile, isotonic solution of Sodium Chloride in Water for Injection. It contains not less than 0.85 per cent and not more than 0.95 per cent w/v of NaCl. It contains no antimicrobial agents.

Description : Clear, colourless solution.

Identification : It gives the reactions of *sodium*, and of *chlorides*, Appendix 3.1.

pH : Between 4.5 and 7.0, Appendix 5.10.

Heavy metals : Not more than 0.3 parts per million, determined by Method A on a solution obtained by evaporating 67 ml to about 20 ml, adding 2 ml of *dilute acetic acid* and then diluting to 25 ml with *water*, Appendix 3.2.4.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using not less than 10 ml per kg of rabbit's weight.

Other requirements : Complies with the requirements stated under Injections.

Assay : Carry out the **Assay** described under Sodium Chloride, using an accurately measured volume equivalent to about 0.25 g of Sodium Chloride. Each ml of *0.1N silver nitrate* is equivalent to 0.005845 g of NaCl.

Storage : Store in single-dose containers of glass or plastic. On keeping, small solid particles may separate from a glass container. A solution containing such particles must not be used.

Labelling : The label on the container of the Injection intended for intravenous infusion states (1) that the Injection contains 150 millimoles of sodium and chloride ions per litre; (2) that the Injection should not be used if it contains visible solid particles.

Sodium Chloride Hypertonic Injection

Hypertonic Saline

Category : Fluid and electrolyte replenisher.

Standards : Sodium Chloride Hypertonic Injection is a sterile solution of Sodium Chloride in Water for Injection. It contains not less than 1.52 per cent and not more than 1.68 per cent w/v of NaCl. It contains no antimicrobial agents.

Description : Clear, colourless solution.

Identification : It gives the reactions of *sodium*, and of *chlorides*, Appendix 3.1.

pH : Between 5.0 and 7.5, Appendix 5.10.

Arsenic : Not more than 0.2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 0.5 parts per million, determined by Method A on a solution prepared by evaporat-

ing 40 ml to about 20 ml, adding 2 ml of *dilute acetic acid* and then diluting to 25 ml with *water*, Appendix 3.2.4.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using a volume equivalent to not less than 0.1 g of Sodium Chloride per kg of the rabbit's weight.

Other requirements : Complies with the requirements stated under Injections.

Assay : Carry out the **Assay** described under Sodium Chloride, using an accurately measured volume equivalent to about 0.25 g of Sodium Chloride. Each ml of *0.1N silver nitrate* is equivalent to 0.005845 g of NaCl.

Storage : Store in single-dose containers of glass or plastic. On keeping, small solid particles may separate from a glass container. A solution containing such particles must not be used.

Labelling : The label on the container of the Injection intended for intravenous infusion states (1) that the injection contains 270 millimoles of sodium and chloride ions per litre; (2) the storage conditions; (3) the date after which the Injection is not intended to be used; (4) that the Injection should not be used if it contains visible solid particles.

Compound Sodium Chloride Injection

Ringer's Injection

Category : Fluid and electrolyte replenisher.

Standards : Compound Sodium Chloride Injection contains not less than 0.82 per cent w/v and not more than 0.90 per cent w/v of NaCl, not less than 0.0285 per cent w/v and not more than 0.0315 per cent w/v of KCl and not less than 0.03 per cent w/v and not more than 0.036 per cent w/v of $CaCl_2,2H_2O$. It contains no antimicrobial agents.

Sodium Chloride	8.6 g
Potassium Chloride	0.3 g
Calcium Chloride	0.33 g
Water for Injection	
sufficient to produce	1000 ml

Dissolve the ingredients in the Water for Injection, filter until clear, and sterilise by *heating in an autoclave.*

Description : Clear, colourless solution.

Identification : It gives the reactions of *sodium*, and of *chlorides*, Appendix 3.1. When concentrated to one half of its original volume, it gives the reactions of *potassium*, and of *calcium*, Appendix 3.1.

pH : Between 5.0 and 7.5, Appendix 5.10.

Arsenic : Not more than 0.1 part per million, Appendix 3.2.1.

Heavy metals : Not more than 0.3 part per million, determined by Method A on a solution prepared by evaporating 67 ml to about 20 ml, adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, Appendix 3.2.4.

Pyrogens : Complies with the *test for pyrogens*, using not less than 10 ml per kg of the rabbit's weight, Appendix 2.36.

Other requirements : Complies with the requirements stated under Injections.

Assay : (1) *For NaCl* – Dilute appropriately with *water* and determine by Method A for *flame photometry*, Appendix 5.16 A, measuring at 589 nm or by Method A for *atomic absorption spectrophotometry*, Appendix 5.16 B, and using *sodium solution FP*, suitably diluted with *water*, for the standard solutions. Each g of Na is equivalent to 2.54 g of NaCl.

(2) *For KCl* – Dilute appropriately with *water*, and determine by Method A for *flame photometry*, Appendix 5.16 A, measuring at 767 or by Method A for *atomic absorption spectrophotometry*, Appendix 5.16 B, and using *potassium solution FP*, suitably diluted with *water*, for the standard solutions. Each g of K is equivalent to 1.007 g of KCl.

(3) *For $CaCl_2, 2H_2O$* – Pipette 50 ml into a beaker, add 5 ml of *0.01M magnesium sulphate* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.01N disodium ethylenediaminetetraacetate* using *mordant black II mixture* as indicator. From the volume of *0.01M disodium ethylenediaminetetraacetate* subtract the volume of *0.01M magnesium sulphate*. Each ml of the remainder is equivalent to 0.001470 g of $CaCl_2, 2H_2O$.

Storage : Store in single-dose containers of glass or plastic. When kept in glass containers for a long time, small solid particles may separate out. A solution containing such particles must not be used.

Labelling : The label on the containers states (1) that the Injection contains in milli equivalent per litre, the following approximate amount of ions: sodium, 147.5; potassium, 4; calcium, 4.5 and chloride, 156; (2) the storage conditions; (3) the date after which the Injection is not intended to be used; (4) that the Injection should not be used if it contains visible solid particles.

Compound Sodium Chloride Solution

Ringer's Solution

Category . Irrigation solution (for external use).

Standards : Compound Sodium Chloride Solution is a sterile solution of Sodium Chloride, Potassium Chloride and Calcium Chloride in Purified Water. It contains not less than 0.82 per cent w/v and not more than 0.90 per cent w/v of NaCl, not less than 0.025 per cent w/v and not more than 0.035 per cent w/v of KCl, not less then 0.03 per cent w/v and not more than 0.036 per cent w/v of $CaCl_2, 2H_2O$.

Description : Clear, colourless solution.

Identification, pH, Arsenic, Heavy metals : Complies with the requirements stated under Compound Sodium Chloride Injection.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Assay : Carry out the **Assay** described under Compound Sodium Chloride Injection.

Storage : Store in tightly-closed containers.

Sodium Chloride and Dextrose Injection

Sodium Chloride and Dextrose Intravenous Infusion

Category : Fluid, nutrient, and electrolyte replenisher.

Usual strengths : Injections containing the following amounts of Sodium Chloride and Dextrose, $C_6H_{12}O_6$.

Percentage of sodium chloride	Percentage of dextrose ($C_6H_{12}O_6$)	Percentage of sodium chloride	Percentage of dextrose ($C_6H_{12}O_6$)
0.11	5		
0.18	5	0.45	5
0.2	5	0.45	10
0.225	5	0.9	2.5
0.3	5	0.9	5
0.33	5	0.9	10
0.45	2.5	0.9	25

Standards : Sodium Chloride and Dextrose Injection is a sterile solution of Sodium Chloride and Dextrose in Water for Injection. It contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of Sodium Chloride, NaCl, and Dextrose, $C_6H_{12}O_6$.

Description : Clear, colourless or faintly straw-coloured solution.

Identification : (A) When heated with *potassium cupritartrate solution*, yields a copious precipitate of cuprous oxide.

(B) A solution (1 in 20) gives the reactions of *sodium*, Appendix 3.1.

(C) When treated with *silver nitrate solution*, yields a curdy white precipitate which is insoluble in *nitric acid* but soluble, after being well washed with *water*, in *dilute ammonia solution*, from which it is reprecipitated by the addition of *nitric acid*.

pH : Between 3.5 and 6.5, Appendix 5.10.

5-Hydroxymethylfurfural and related substances : Dilute a volume equivalent to 1 g of dextrose, $C_6H_{12}O_6$ to 500.0 ml with *water* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 284 nm, Appendix 5.15 A, the *extinction* is not more than 0.25.

Heavy metals : Complies with the test for **Heavy metals** described under Dextrose Injection.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using not less than 10 ml per kg of the rabbit's weight (Injections containing more than 10 per cent of dextrose should be diluted with *water for injection* to give a concentration of 10 per cent of dextrose, $C_6H_{12}O_6$).

Other requirements : Complies with the requirements stated under Injections.

Assay : *For sodium chloride* – Titrate an accurately measured volume equivalent to about 0.1 g of Sodium Chloride with *0.1N silver nitrate*, using *potassium chromate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.005845 g of NaCl.

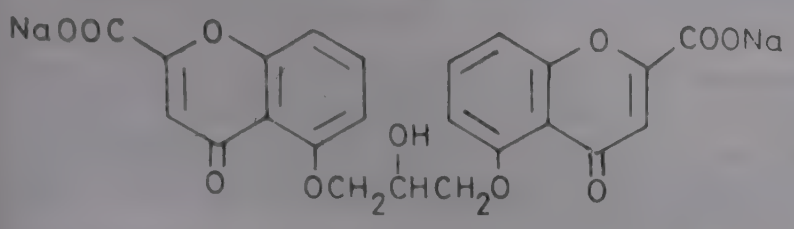
For dextrose – Carry out the **Assay** described under Dextrose Injection.

Storage : Store in single-dose containers in a cool place. On keeping, there may be separation of small solid particles.

Labelling : The label on the container states (1) the strength as the percentages w/v of Sodium Chloride and of Dextrose, $C_6H_{12}O_6$; (2) the approximate concentrations, in milli equivalents per litre, of the sodium ions and the chloride ions (when the preparation is intended for intravenous infusion); (3) that

the injection should not be used if it contains visible solid particles.

Sodium Cromoglycate



$C_{23}H_{14}Na_2O_{11}$ Mol. Wt. 512.34

Category : Prophylactic in asthma.

Dose : By inhalation, 20 mg, every 4 to 6 hrs.

Description : White, crystalline powder; odourless; tasteless at first, becoming slightly bitter; hygroscopic.

Solubility : Freely soluble in *water*; insoluble in *alcohol*, and in *chloroform*.

Standards : Sodium Cromoglycate is disodium 4, 4'-dioxo-5, 5'-(2-hydroxy-trimethylenedioxy)di(chromene-2-carboxylate). It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{23}H_{14}Na_2O_{11}$, calculated with reference to the dried substance.

Identification : (A) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *buffer solution, pH 7.4* exhibits two maxima, at 238 nm and at 326 nm, *extinction* at 238 nm about 0.6, and at 326 nm about 0.16, Appendix 5.15 A.

(B) To 0.1 g add 2 ml of *water* and 2 ml of *dilute sodium hydroxide solution* and boil for one minute; a yellow solution is obtained. To the hydrolysed solution add 0.5 ml of *diazo-benzenesulphonic acid solution*; a blood-red colour is produced.

(C) A solution (1 in 10) gives the reactions of *sodium*, Appendix 3.1.

Acidity or Alkalinity : Dissolve 1.0 g in 25 ml of freshly boiled and cooled *water*, add a few drops of *bromothymol blue solution*; the solution requires for neutralisation not more than 0.25 ml of *0.1N sodium hydroxide* or *0.1N hydrochloric acid*.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 3.2.4.

Oxalate : Dissolve 0.10 g in 20 ml of *water*, add 5.0 ml of *iron salicylate solution* and sufficient *water* to produce 50 ml. Measure the *extinction* of a 1-cm layer of the resulting solution at 480 nm, Appendix 5.15 A. The *extinction* is not less than that obtained by repeating the operation using 0.35 mg of *oxalic acid* instead of the sodium cromoglycate.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel GF 254* as coating substance, omitting the heating, and allowing the plate to stand overnight at room temperature. Use as the mobile phase a mixture of 9 volumes of *chloroform*, 9 volumes of *methyl alcohol*, and 2 volumes of *glacial acetic acid*. Apply separately to the plate 10 µl of each of two solutions, in a mixture of 1 volume of *acetone*, 4 volumes of *tetrahydrofuran* (which has been freed from stabiliser by passage through a column of suitable alumina), and 6 volumes of *water*, containing (1) 2.0 per cent w/v of the substance being examined and (2) 0.01 per cent w/v of the substance being examined. After removal of the plate dry it in a current of warm air, and examine under an ultra-violet lamp having a maximum output at about 254 nm. Any spot in the chromatogram obtained with solution (1) moving ahead of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying : Not more than 10.0 per cent, determined on 1.0 g by drying "in vacuo at 100°", Appendix 5.8.

Assay : Weigh accurately about 0.1 g and dissolve in sufficient quantity of *buffer solution, pH 7.4* to produce 1000.0 ml. Dilute 25.0 ml to 100.0 ml with *buffer solution, pH 7.4* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum of about 326 nm, Appendix 5.15 A. Calculate the content of $C_{23}H_{14}Na_2O_{11}$, from the *extinction* obtained by carrying out the assay simultaneously using about 0.1 g, accurately weighed, of *sodium cromoglycate R.S.*

Storage : Store in well-closed containers.

Sodium Cromoglycate Cartridges

Category : Prophylactic in asthma.

Dose : By inhalation, 20 mg every 4 to 6 hours.

Usual strength : 20 mg.

Standards : Sodium Cromoglycate Cartridges are capsules of hard gelatin or any other suitable material, containing a mixture of approximately equal parts of Sodium Cromoglycate in powder of a suitable fineness and Lactose. The cartridges contain not less than 100.0 per cent and not more than 120.0 per cent of the stated amount of sodium cromoglycate, $C_{23}H_{14}Na_2O_{11}$.

Identification : The contents of the cartridges comply with **Identification** tests (A), (B) and (C) described under Sodium Cromoglycate.

Related substances : The contents of the cartridges comply with the test described under Sodium Cromoglycate, applying to the plate 10 µl of each of two solutions prepared in the following manner: For solution (1) dissolve a quantity equivalent to 0.10 g of Sodium Cromoglycate in sufficient quantity of a mixture of 1 volume of *acetone*, 4 volumes of *tetrahydrofuran* (which has been freed from stabilizer by passage through a column of suitable alumina), and 6 volumes of *water* to produce 5 ml and filter; for solution (2) dilute 1 volume of solution (1) to 200 volumes with the same solvent.

Other requirements : Comply with the requirements stated under Capsules. The requirement for Disintegration does not apply to Sodium Cromoglycate Cartridges.

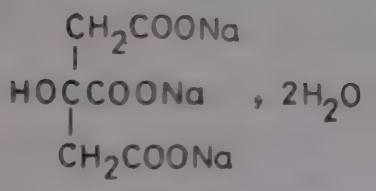
Loss on drying : Not less than 5.5 per cent and not more than 10.0 per cent, determined on 0.5 g of the contents of the cartridges, by drying "in vacuo at 100°", Appendix 5.8.

Assay : Weigh accurately a portion of the mixed contents of twenty capsules equivalent to about 0.1 g of Sodium Cromoglycate and dissolve in sufficient *buffer solution, pH 7.4* to produce 1000.0 ml. Dilute 25.0 ml of this solution to 100.0 ml with *buffer solution, pH 7.4* and measure the *extinction* of 1-cm layer of the resulting solution at the maximum at about 326 nm, Appendix 5.15 A. Calculate the content of $C_{23}H_{14}Na_2O_{11}$, taking 164 as the value of E(1 per cent, 1-cm) at the maximum at about 326 nm.

Storage : Store in well-closed containers, protected from moisture at a temperature not exceeding 30°

Labelling : The label on the container states (1) the strength in terms of equivalent amount of Sodium Cromoglycate; (2) that the cartridges are intended for use in an inhaler and are not to be swallowed.

Sodium Citrate



$C_6H_5Na_3O_7, 2H_2O$ Mol. Wt. 294.10

Category : Systemic alkaliser.

Dose : 1 to 10 g.

Description : Colourless crystals or white crystalline powder; odourless; taste, saline; slightly deliquescent in moist air.

Solubility : Very soluble in boiling *water*; freely soluble in *water*; practically insoluble in *alcohol (90 per cent)*, and in *solvent ether*.

Standards : Sodium Citrate is dihydrate of trisodium 2-hydroxypropane-1,2,3-tricarboxylate. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_6H_5Na_3O_7$, calculated with reference to the anhydrous substance.

Identification : A solution (1 in 20) gives the *reactions* of *sodium*, and of *citrates*, Appendix 3.1.

Acidity or Alkalinity : Boil 2 g with 25 ml of *water* and cool; titrate with *0.1N sulphuric acid* or *0.1N sodium hydroxide*, using *thymol blue solution* as indicator; not more than 0.5 ml of *0.1N sulphuric acid* or *0.1N sodium hydroxide* is required.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined by Method A on a solution prepared by dissolving 2 g in 10 ml of *water*, 5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml, Appendix 3.2.4.

Chloride : 1 g dissolved in *water* with the addition of 2 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 0.5 g dissolved in *water* with the addition of 2 ml of *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

Tartrate : To a solution of 1 g in 2 ml of *water* in a test-tube, add 1 ml of a 10 per cent w/v solution of *potassium acetate* and 1 ml of *6N acetic acid*. Rub the walls of the test-tube with a glass rod; no cyrstalline precipitate is formed.

Oxalate : Dissolve 1 g in a mixture of 1 ml of *water* and 3 ml of *dilute hydrochloric acid*, add 4 ml of *alcohol (90 per cent)* and four drops of *calcium chloride solution* and allow to stand for one hour; the mixture remains clear.

Readily carbonisable substances : Heat 0.2 g in a boiling water-bath for one hour with 10 ml of *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4); the solution is not more intensely coloured than a mixture of 1.5 ml of *ferric chloride C.S.*, 0.3 ml of *cobalt chloride C.S.*, 0.3 ml of *copper sulphate C.S.*, and sufficient *water* to produce 10 ml.

Water : Between 11.0 per cent w/w and 13.0 per cent w/w, Appendix 3.3.25.

Assay : Weigh accurately about 0.25 g and dissolve in 50 ml of *glacial acetic acid* by heating to about 50°. Cool and titrate with *0.1N perchloric acid*, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.008602 g of $C_6H_5Na_3O_7$.

Storage : Store in tightly-closed containers.

Sodium Hydroxide

NaOH Mol. Wt. 40.00

Category : Pharmaceutical aid (alkalising agent).

Description : White sticks, pellets, fused masses, or scales; dry, hard brittle, and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

Solubility : Freely soluble in *water* and in *alcohol*.

Standards : Sodium Hydroxide contains not less than 95.0 per cent of total alkali, calculated as NaOH, including not more than 3.0 per cent of Na_2CO_3.

Identification : A solution (1 in 20) gives the reactions of *sodium*, Appendix 3.1.

Insoluble substances and organic matter : A 5 per cent w/v solution is clear and colourless.

Aluminium, iron and matter insoluble in hydrochloric acid : Boil 5 g with 50 ml of *dilute hydrochloric acid*. Cool, make alkaline with *dilute ammonia solution*, boil, filter, and wash with a 2.5 per cent w/v solution of *ammonium nitrate*; the insoluble residue after ignition to constant weight weighs not more than 5 mg.

Arsenic : Not more than 4 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 30 parts per million, determined by Method A, Appendix 3.2.4, on a solution prepared by dissolving 0.67 g in 5 ml of *water* and 7 ml of *3N hydrochloric acid*. Heat to boiling, cool and dilute to 25 ml with *water*.

Potassium : Acidify 5 ml of a 5 per cent w/v solution with *acetic acid* and add 3 drops of *sodium cobaltinitrite solution*; no precipitate is formed.

Chloride : 0.5 g dissolved in *water* with the addition of 1.8 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphates : 1 g dissolved in *water* with the addition of 3.5 ml of *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

Assay : Weigh accurately about 1.5 g and dissolve in about 40 ml of *carbon dioxide-free water*. Cool and titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. When the pink colour of the solution is discharged, record the volume of acid solution required, add *methyl orange solution* and continue the titration until a persistent pink colour is produced. Each ml of *N sulphuric acid* is equivalent to 0.040 g of total alkali, calculated as NaOH and each ml of acid consumed in the titration with methyl-orange is equivalent to 0.106 g of Na_2CO_3.

Storage : Store in tightly-closed containers.

Sodium Lactate Injection

Sodium Lactate Intravenous Infusion

Category : Fluid and electrolyte replenisher.

Dose : By intravenous infusion, as a one-sixth molar solution, at the rate of 5 ml or less per minute.

Usual strength : One-sixth molar.

Description : Clear, colourless solution.

Standards : Sodium Lactate Injection contains not less than 1.75 per cent w/v and not more than 1.95 per cent w/v of $C_3H_5NaO_3$.

Lactic Acid	14.0 ml
Sodium Hydroxide	6.7 g
Dilute Hydrochloric Acid	a sufficient quantity
Water for Injection, sufficient to produce	1000 ml

Dissolve the Sodium Hydroxide in 400 ml of Water for Injection, add the Lactic Acid, and heat in an autoclave at 115° to 116° for one hour. Cool and carefully add Dilute Hydrochloric Acid until 0.15 ml of the solution gives a full orange colour with 0.05 ml of *phenol red solution*. Add sufficient Water for Injection to produce 1000 ml. Filter and immediately sterilise by *heating in an autoclave*.

Identification : (A) When warmed with *potassium permanganate*, yields acetaldehyde, recognisable by its odour.

(B) The residue on evaporation gives the reactions of *sodium*, Appendix 3.1.

pH : Between 5.0 and 7.0, Appendix 5.10.

Heavy metals : Not more than 10 parts per million, determined by Method A on a solution prepared by evaporating 110 ml to 20 ml adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, Appendix 3.2.4.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using not less than 10 ml per kg of the rabbit's weight.

Other requirements : Complies with the requirements stated under Injections.

Assay : Measure accurately 10 ml, evaporate to dryness in a platinum dish and ignite very gently until completely carbonised. Boil the residue with 25.0 ml of *0.1N sulphuric acid*, filter, and wash thoroughly with hot *water*. Titrate the excess of acid in the combined filtrate and washings with *0.1N sodium hydroxide* using *methyl orange solution* as indicator. Each ml of *0.1N sulphuric acid* is equivalent to 0.01121 g of $C_3H_5NaO_3$.

Storage : Store in single-dose containers of glass or plastic. On keeping, there may be separation of small solid particles.

Labelling : The label on the container states (1) that the injection is one-sixth molar and contains approximately 167 millimoles of sodium and lactate per litre; (2) the date after which the Injection is not intended to be used; (3) the storage conditions; (4) that the Injection should not be used if it contains visible particles.

Compound Sodium Lactate Injection

Ringer Lactate Solution for Injection, Hartmann's Solution for Injection.

Category : Fluid and electrolyte replenisher.

Standards : Compound Sodium Lactate Injection contains not less than 0.37 per cent w/v and not more than 0.42 per cent w/v of total Cl, not less than 0.025 per cent w/v and not more than 0.029 per cent w/v of $CaCl_2, 2H_2O$ and not less than 0.23 per cent w/v and not more than 0.28 per cent w/v of lactic acid, $C_3H_6O_3$. It contains no antimicrobial agents.

Lactic Acid	2.4 ml
Sodium Hydroxide	1.15 g
Dilute Hydrochloric Acid	a sufficient quantity
Sodium Chloride	6 g
Potassium Chloride	0.4 g
Calcium Chloride	0.27 g
Water for Injection sufficient to produce	1000 ml

Dissolve the Sodium Hydroxide in 200 ml of Water for Injection; add the Lactic Acid, and heat in an autoclave at 115° to 116° for one hour. Cool and cautiously add Dilute Hydrochloric Acid (about 1 ml is normally required) until 0.15 ml of the solution gives a full orange colour with 0.05 ml of *phenol red solution*. Dissolve the other ingredients in 700 ml of Water for Injection, mix the two solutions, and add sufficient Water for Injection to produce 1000 ml. Filter and immediately sterilise.

Description : Clear, colourless solution.

Identification : (1) When warmed with *potassium permanganate*, yields acetaldehyde, recognisable by its odour.

(2) It gives the reactions of *calcium*, Appendix 3.1.

(3) The residue obtained by evaporation gives after incineration, the reactions of *potassium*, and of *sodium*, Appendix 3.1.

pH : Between 5.0 and 7.0, Appendix 5.10.

Heavy metals : Not more than 0.3 parts per million, determined by Method A on a solution prepared by evaporating 67 ml to a volume of 20 ml, adding 2 ml of *dilute acetic acid* and then diluting to 25 ml with *water*, Appendix 3.2.4.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using not less than 10 ml per kg of the rabbit's weight.

Other requirements : Complies with the requirements stated under Injections.

Assay : (1) *For total Cl* – To 20 ml add 30 ml of *water*, 50 ml of *0.1N silver nitrate*, and 2 ml of *nitric acid*, filter, wash the precipitate with *water* slightly acidified with *nitric acid*, and titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.003545 g of total Cl.

(2) *For $CaCl_2, 2H_2O$* – To 50 ml add 5 ml of *0.01N magnesium sulphate* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.01M disodium ethylenediaminetetraacetate*, using *mordant black II mixture* as indicator. From the volume of *0.01M disodium ethylenediaminetetraacetate* required, subtract the volume of *0.01M magnesium sulphate* added. Each ml of the remainder is equivalent to 0.001470 g of $CaCl_2, 2H_2O$.

(3) *For $C_3H_6O_3$* – Evaporate 50 ml to dryness in a platinum dish and ignite very gently until completely carbonised. Boil the residue with 25 ml of *0.1N sulphuric acid*, filter, and wash thoroughly with hot *water*. Titrate the excess of acid in the combined filtrate and washings with *0.1N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *0.1N sulphuric acid* is equivalent to 0.009008 g of $C_3H_6O_3$.

Storage : Store in single-dose containers of glass or plastic.

CAUTION – Small solid particles may separate from a glass container on keeping. A solution containing such particles must not be used.

Labelling : The label on the containers states (1) that the injection contains, in milliequivalent per litre, the following approximate amounts of ions; sodium, 131; potassium, 5; calcium, 4; bicarbonate (as lactate), 29; and chloride, 111; (2) the storage

conditions; and (3) the date after which the Injection is not intended to be used.

Sodium Lauryl Sulphate

Category : Pharmaceutical aid (surfactant).

Description : White or pale-yellow crystals or crystalline powder; odour, slight but characteristic.

Solubility : Soluble in *water* forming an opalescent solution; partly soluble in *alcohol*.

Standards : Sodium Lauryl Sulphate is a mixture of sodium normal primary alkyl sulphates, consisting chiefly of sodium dodecyl sulphate, $C_{12}H_{25}OSO_3Na$. It contains not less than 85.0 per cent of sodium alkyl sulphates, calculated as $C_{12}H_{25}OSO_3Na$.

Identification : (A) A 1 per cent w/v solution, when shaken, produces plenty of foam.

(B) Mix 0.1 ml of a 1 per cent w/v solution with 0.1 ml of a 0.1 per cent w/v solution of *methylene blue* and 2 ml of *2N sulphuric acid*. Add 2 ml of *chloroform* and shake; the chloroform layer is coloured dark blue.

(C) A solution (1 in 20) gives the reactions of *sodium*, and of *sulphates*, Appendix 3.1.

Alkalinity : Dissolve 1 g in 100 ml of *water*; add a few drops of *phenol red solution* and titrate with *0.1N hydrochloric acid*; not more than 0.5 ml is required.

Sodium chloride and sodium sulphate : Not more than 10.0 per cent of NaCl and Na_2SO_4, taken together when determined by the following methods:

(a) Sodium chloride : Weigh accurately about 5 g and dissolve in 50 ml of *water*, add *dilute nitric acid*, drop by drop, until the solution is neutral to *litmus paper*, add 2 ml of *potassium chromate solution* and titrate with *0.1N silver nitrate*; each ml of *0.1N silver nitrate* is equivalent to 0.005845 g of NaCl.

(b) Sodium sulphate : Weigh accurately about 1 g and place in 500-ml beaker, add 10 ml of *water*, and heat until solution is complete. To the hot solution add 100 ml of *alcohol*, cover and digest on a water-bath for two hours at a temperature just below its boiling point. Filter, while hot, through a Gooch crucible and wash with 100 ml of hot alcohol. Dissolve the precipitate in 100 ml of *water* containing 10 ml of *hydrochloric acid*, heat to boiling, add 25 ml of *barium chloride solution*, and allow to stand overnight. Filter off the precipitate, wash with water until the washings give no reactions for chloride, dry and ignite. Each g of residue is equivalent to 0.6085 g of Na_2SO_4.

Non-esterified alcohols : Dissolve 10 g in 100 ml of *Water*, add 100 ml of *alcohol*, and extract the solution with three successive quantities, each of 50 ml, of *light petroleum (boiling range 40° to 60°)*, add *sodium chloride* if necessary, to promote separation of two layers. Wash the mixed extracts with three quantities, each of 50 ml, of *water*, dry the solution with *anhydrous sodium sulphate*, filter into a tared beaker and evaporate on a water-bath until the odour of light petroleum is no longer perceptible. Heat the residue at 105° for thirty minutes and cool; the residue weighs not more than 0.4 g.

Assay : Dissolve 1.15 g in sufficient *water* to produce 1000.0 ml. To 20.0 ml add 10 ml of *water*, 15 ml of *chloroform*, 10 ml of *dilute sulphuric acid* and 1 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.004M benzethonium chloride*, shaking vigorously and allowing the layers to separate after each addition; until the chloroform layer acquires a permanent clear green colour. Each ml of *0.004M benzethonium chloride* is equivalent to 0.001154 g of sodium alkyl sulphates, calculated as $C_{12}H_{25}OSO_3Na$.

Storage : Store in well-closed containers.

Sodium Metabisulphite

$Na_2S_2O_5$ Mol. Wt. 190.10

Category : Pharmaceutical aid (anti-oxidant).

Description : Colourless, prismatic crystals or white powder which may become yellowish on keeping; odour, sulphurous; taste, acid and saline.

Solubility : Freely soluble in *water*; slightly soluble in *alcohol*.

Standards : Sodium Metabisulphite contains not less than 95.0 per cent of $Na_2S_2O_5$.

Identification : A solution (1 in 20) complies with the following:

(i) It decolourises *iodine solution*, and the resulting solution gives the reactions of *sulphates*, Appendix 3.1.

(ii) It gives the reactions of *sodium*, Appendix 3.1.

Acidity : A solution is acid to *phenol red solution*.

Arsenic : Not more than 4 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined by Method A on a solution prepared in the following manner: Dissolve 1 g in 10 ml of *water*, add 5 ml of *hydrochloric acid* and evaporate on a water-bath to dryness. Dissolve the residue in 25 ml of *water* containing 2 ml of *dilute acetic acid*, Appendix 3.2.4.

Iron : To 0.5 g add 2 ml of *hydrochloric acid* and evaporate on a water-bath to dryness. Dissolve the residue in 2 ml of *hydrochloric acid* and 20 ml of *water* and add a few drops of *bromine solution*. Boil the solution to remove the bromine, cool, dilute with *water* to 25 ml, then add 50 mg of *ammonium persulphate* and 5 ml of *ammonium thiocyanate solution*; any colour produced is not darker than that obtained by adding 5 ml of *ammonium thiocyanate solution* to a mixture of 1.0 ml of *standard iron solution*, 2 ml of *hydrochloric acid*, 50 mg of *ammonium persulphate* and sufficient *water* to produce 25 ml.

Thiosulphate : Mix 2.2 g with 10 ml of *N hydrochloric acid*. Boil gently for five minutes, cool, and transfer to a small test-tube. Any turbidity is not greater than that produced by 0.1 ml of *0.1N sodium thiosulphate*, similarly treated.

Assay : Weigh accurately about 0.2 g and dissolve in 50.0 ml of *0.1N iodine*, add 1 ml of *hydrochloric acid* and titrate the excess of iodine with *0.1N sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Each ml of *0.1N iodine* is equivalent to 0.004753 g of $Na_2S_2O_5$.

Storage : Store in tightly-closed containers.

Sodium Phosphate

Disodium Hydrogen Phosphate

$Na_2HPO_4, 12H_2O$ Mol. Wt. 358.14

Category : Cathartic; pharmaceutical aid (buffering agent).

Description : Colourless, transparent crystals; odourless. Strongly efflorescent.

Solubility : Freely soluble in *water*; practically insoluble in *alcohol*.

Standards : Sodium Phosphate is the dodecahydrate of disodium hydrogen orthophosphate. It contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of Na_2HPO_4, calculated with reference to the dried substance.

Identification : A 10 per cent w/v solution gives the reactions of *sodium* and of *phosphates*, Appendix 3.1.

Clarity and colour of solution : A 10.0 per cent w/v solution is clear and colourless.

Arsenic : Not more than 5 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined by Method A on a solution prepared by dissolving 2 g in 10 ml of *water*, adding 4 ml of *N acetic acid* and diluting to 25 ml with *water*, Appendix 3.2.4.

Iron : 0.5 g complies with the *limit test for iron*, Appendix 3.2.5.

Chloride : Dissolve 0.25 g in 10 ml of *2N nitric acid* and dilute to 15 ml with *water*; the resulting solution complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : Dissolve 0.15 g in 2 ml of *2N nitric acid* and dilute to 15 ml with *water*; the resulting solution complies with the *limit test for sulphates*, Appendix 3.2.8.

Reducing substances : Dissolve 0.5 g in 10 ml of *N sulphuric acid* and 0.25 ml of *0.1N potassium permanganate*, and heat on a water-bath for five minutes; the red colour is not completely discharged.

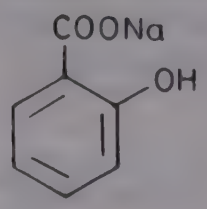
Monosodium phosphate : The value n_2 obtained in the **Assay** does not exceed the value $(n - n_1)$ by more than 5 per cent.

Loss on drying : Between 57.0 per cent and 61.0 per cent, determined on 0.5 g, by drying in an oven at 130°, taking care to avoid sputtering, Appendix 5.8.

Assay : Weigh accurately about 4 g (w g) in 25.0 ml of *N hydrochloric acid* (n ml) and dilute to 100.0 ml with *water*. Titrate potentiometrically 10.0 ml of the solution with *0.1N sodium hydroxide* until pH 4.4 is reached (n_1 ml). To the titrated solution add 4 g of *sodium chloride* and continue the titration with *0.1N sodium hydroxide* to pH 9.2 (n_2 ml). Determine the percentage content of Na_2HPO_4 from the expression $1420(n_2 - n_1)/w(100-d)$, where d is the percentage loss on drying.

Storage : Store in tightly-closed containers.

Sodium Salicylate



$C_7H_5NaO_3$ Mol. Wt. 160.10

Category : Analgesic, antipyretic.

Dose : 0.6 to 2.0 g. In the treatment of acute rheumatism, 5 to 10 g daily, in divided doses.

Description : Colourless, small crystals; or white flakes, or crystalline powder; odourless or with faint characteristic odour; taste, sweetish, saline and unpleasant.

Solubility : Very soluble in *water*; soluble in *alcohol*, practically insoluble in *chloroform* and *solvent ether*.

Standards : Sodium Salicylate is monosodium 2-hydroxybenzoate. It contains not less than 99.5 per cent and not more than the equivalent of 101.0 per cent of $C_7H_5NaO_3$, calculated with reference to the dried substance.

Identification : 5.0 per cent w/v solution complies with the following tests:

(i) It gives the reactions of *sodium*, Appendix 3.1.

(ii) To 1 ml add 4 ml of *water* and one drop of *ferric chloride test-solution*; a violet colour is produced.

(iii) To 5 ml add 1 ml of *dilute sulphuric acid*; a white precipitate is produced which after washing with *water* and drying has a melting range between 158° and 161°, Appendix 5.11.

Acidity : To 20 ml of a 10.0 per cent w/v solution add two drops of *phenol red solution*; the solution is yellow. Titrate with *0.1N sodium hydroxide* to a red-violet colour. Not more than 0.2 ml of *0.1N sodium hydroxide* is required.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined by Method A, on a solution prepared by dissolving 2.0 g in 46 ml of *water* adding with constant stirring 4 ml of *dilute hydrochloric acid*, filtering and using 25 ml of the filtrate, Appendix 3.2.4.

Chlorides : Dissolve 1 g in 40 ml of *water*, add 1 ml of *nitric acid* and filter; the filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphates : Dissolve 1 g in 50 ml of *water*, add 1 ml of *hydrochloric acid*, and filter; the filtrate complies with the *limit test for sulphates*, Appendix 3.2.8.

Loss on drying : Not more than 1.0 per cent determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.1 g in a glass-stoppered flask and dissolve in 50 ml of *water*. Add 25.0 ml of *0.2N potassium bromate*, 1 g of *potassium bromide* and 10 ml of *dilute hydrochloric acid* and allow to stand for fifteen minutes. Add 1 g of *potassium iodide* and allow to stand for ten minutes. Titrate with *0.1N sodium thiosulphate*, shaking vigorously. Each ml of *0.2N potassium bromate* is equivalent to 0.005336 g of $C_7H_5NaO_3$.

Storage : Store in well-closed light-resistant containers.

Sodium Thiosulphate

$Na_2S_2O_3, 5H_2O$ Mol. Wt. 248.17

Category : Antidote to cyanide poisoning.

Dose : By intravenous or intramuscular injection, 25 g.

Description : Large colourless crystals or coarse, crystalline powder; odourless; taste, saline. Deliquescent in moist air and effloresces in dry air at temperature above 33°.

Solubility : Very soluble in *water*; insoluble in *alcohol*.

Standards : Sodium Thiosulphate contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $Na_2S_2O_3, 5H_2O$.

Identification : (A) To a 10 per cent w/v solution and a few drops of *iodine solution*; the colour is discharged.

(B) A solution (1 in 20) gives the reactions of *sodium*, and of *thiosulphates*, Appendix 3.1.

pH : Between 6.0 and 8.4, determined in a 10 per cent w/v solution, Appendix 5.10.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined by Method A, Appendix 3.2.4., on a solution prepared in the following manner: Dissolve 1 g in 10 ml of *water*. Slowly add 5 ml of *dilute hydrochloric acid* and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of *water* for two minutes, and filter. Heat the filtrate to boiling, and add sufficient *bromine solution* to the hot filtrate to produce a clear solution and add a slight excess of *bromine solution*. Boil the solution to expel the bromine completely, cool to room temperature, then add a drop of *phenolphthalein solution* and *sodium hydroxide solution* until a slight pink colour is produced. Add 2 ml of *dilute acetic acid* and dilute with *water* to 25 ml.

Calcium : Dissolve 1 g in 20 ml of *water*, and add a few ml of *ammonium oxalate solution*; no turbidity is produced.

Chloride : Dissolve 0.25g in 15 ml of *2N nitric acid* and boil gently for three to four minutes; cool and filter; the filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate and sulphite : Dissolve 0.25 g in 10 ml of *water*; to 3 ml of this solution add 2 ml of *iodine solution*, and gradually add more *iodine solution* dropwise until a very faint-persistent yellow colour is produced; the resulting solution complies with the *limit test for sulphates*, Appendix 3.2.8.

Sulphide : Dissolve 1 g in 10 ml of *water*, add 0.05 ml of

a freshly prepared 5 per cent w/v solution of *sodium nitroprusside*; the solution does not become violet.

Assay : Weigh accurately about 0.8 g and dissolve in 30 ml of *water*. Titrate with *0.1N iodine*, using 3 ml of *starch solution* as indicator as the end-point is approached. Each ml of *0.1N iodine* is equivalent to 0.02482 g of $Na_2S_2O_3, 5H_2O$.

Storage : Store in tightly-closed containers.

Sodium Thiosulphate Injection

Category : Antidote to cyanide poisoning.

Dose : Sodium thiosulphate; by intramuscular or intravenous injection, 0.3 to 1 g.

Usual strength : 100 mg per ml.

Standards : Sodium Thiosulphate Injection is a sterile solution of Sodium Thiosulphate in freshly-boiled Water for Injection. It contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $Na_2S_2O_3,5H_2O$.

Description : Clear, colourless solution.

Identification : Complies with the **Identification** test described under Sodium Thiosulphate.

pH : Between 8.0 and 9.5, Appendix 5.10.

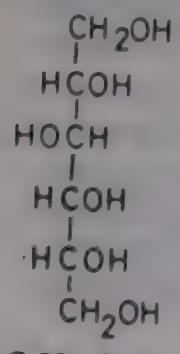
Other requirements : Complies with requirements stated under Injections.

Assay : Carry out the **Assay** described under Sodium Thiosulphate, using an accurately measured volume equivalent to about 0.75 g of Sodium Thiosulphate and adjust the pH to 7.0, if necessary, by the addition of *dilute hydrochloric acid*. Each ml of *0.1N iodine* is equivalent to 0.02482 g of $Na_2S_2O_3, 5H_2O$.

Storage : Store in single-dose containers.

Sorbitol

D-Glucitol



$C_6H_{14}O_6$ Mol. Wt. 182.17

Category : Pharmaceutical aid (sweetening agent, tablet excipient).

Description : White, microcrystalline powder, granules or flakes; odourless; taste, sweetish; hygroscopic.

Solubility : Freely soluble in *water*; slightly soluble in *alcohol* in *methyl alcohol* and in *acetic acid*; insoluble in *chloroform* and in *solvent ether*.

Standards : Sorbitol contains not less than 95.0 per cent and not more than the equivalent of 101.0 per cent of $C_6H_{14}O_6$, calculated with reference to the anhydrous substance.

Identification : (A) Dissolve 50 mg in 3 ml of *water*, add 3 ml of *catechol solution*, and pour the mixture into 6 ml of *sulphuric acid*; a pink colour is produced.

(B) Dissolve 5 g in 3 ml of *water* with the aid of gentle heat, cool, add 7 ml of *methyl alcohol*, 1 ml of *benzaldehyde* and 1 ml of *hydrochloric acid*, mix, and shake continuously for two hours. Filter, dissolve the crystals in 20 ml of boiling *sodium bicarbonate solution*, and allow to crystallise. The residue, after washing rapidly with 5 ml of a mixture of equal volumes of *methyl alcohol* and *water* and drying in a current of air, melts at about 175°, Appendix 5.11.

(C) It melts at about 95°, Appendix 5.11.

Clarity and colour of solution : Dissolve 10 g in 50 ml of *water*; the solution is clear and the colour is not deeper than that of 50 ml of a solution prepared by mixing 0.5 ml of *cobalt chloride C.S.*, 1.2 ml of *ferric chloride C.S.*, and 0.2 ml of *copper sulphate C.S.* and 7.0 ml of *hydrochloric acid* with sufficient *water* to produce 200.0 ml.

pH : Between 6.0 and 7.0, determined in a 10 per cent w/v solution, Appendix 5.10.

Specific optical rotation : Between +13° and +16°, determined in a solution prepared by dissolving 5 g with 15 g *borax* in sufficient *N sodium hydroxide* to produce 100.0 ml, Appendix 5.12.

Chloride : 5.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 5.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 3.2.4.

Nickel : Not more than 1 part per million, determined by the following method: Dissolve 10 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml

of *6N ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *alcohol*. Mix, dilute to 50 ml with *water* and allow to stand for five minutes; any colour produced is not more intense than that produced by similarly treating, at the same time, 1 ml of *standard nickel solution* diluted to 20 ml with *water*.

Reducing sugars : Dissolve 2.5 g in 3 ml of *water* with the aid of gentle heat, cool, add 20 ml of *copper reagent* and heat in such a manner that the solution boils in four minutes, and continue boiling for a further three minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* followed by 20.0 ml of *0.05N iodine*. Add, with continuous swirling, 25 ml of a 6.0 per cent v/v solution of *hydrochloric acid*, and when any precipitate has redissolved, titrate the excess of iodine with *0.05N sodium thiosulphate*, using *starch solution*, added towards the end of the titration as indicator. Not less than 12.8 ml of *0.05N sodium thiosulphate* is required,

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Water : Not more than 1.5 per cent w/w, Appendix 3.3.25.

Assay : Carry out the **Assay**, described under Mannitol.

Storage : Store in tightly-closed containers.

Labelling : The label on the container states that the contents are not intended for parenteral use.

Sorbitol Solution

Category : Pharmaceutical aid (sweetened vehicle); humectant.

Description : Clear, colourless or faintly yellow syrupy liquid; odourless; taste, sweet.

Standards : Sorbitol Solution is a solution containing not less than 68.0 per cent and not more than 72.0 per cent w/w of total alcohols calculated as $C_6H_{14}O_6$ and consisting mainly of sorbitol and small amounts of mannitol and other isomeric polyhydric alcohols.

Identification : (A) Mix 0.1 g with 7.5 ml of *water*; to 3 ml of the mixture in a test-tube, add 3 ml of a freshly prepared 10 per cent w/v solution of *catechol*, mix, add 6 ml of *sulphuric acid* and again mix. Heat gently over a flame for about thirty seconds; a faint pink colour develops.

(B) To 6 ml add 7 ml of *methyl alcohol*, 1 ml of *benzaldehyde* and 1 ml of *hydrochloric acid*, and shake in a mechanical shaker until crystals appear. Filter with suction, dissolve the crystals in 20 ml of boiling *water* containing 1 g of *sodium bicarbonate*, filter while hot, cool the filtrate, filter with suction, wash with 5 ml of a mixture of equal parts of *methyl alcohol* and *water*, and dry in air. The residue so obtained melts at about 175°, Appendix 5.11.

Specific gravity : Not less than 1.285, Appendix 5.19.

Refractive index : Between 1.455 and 1.465, determined at 20°, Appendix 5.14.

pH : Between 6.0 and 7.0, Appendix 5.10.

Chlorides : 7.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphates : 7.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Arsenic : Not more than 3 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 3.2.4.

Reducing sugars : On 3.5 g carry out the test described under Sorbitol, beginning at the words "add 20 ml....".

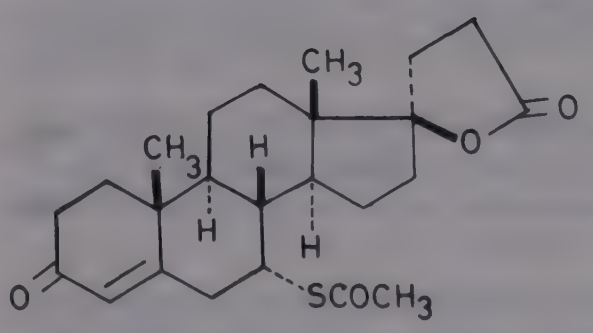
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Water : Between 29.0 per cent and 31.0 per cent w/w, Appendix 3.3.25.

Total alcohols : Weigh accurately about 0.6 g, dilute to 100.0 ml with *water* and complete the **Assay** described under Mannitol, beginning at the words "transfer 10.0 ml to a stoppered flask....".

Storage : Store in tightly-closed containers.

Spironolactone



$C_{24}H_{32}O_4S$ Mol. Wt. 416.57

Category : Aldosterone inhibitor (diuretic).

Dose : 100 to 200 mg daily in divided doses.

Description : Buff coloured powder; odourless, or with a slight odour of thioacetic acid.

Solubility : Practically insoluble in *water*; slightly soluble in *alcohol* and in *solvent ether*; freely soluble in *chloroform*.

Standards : Spironolactone in 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone. It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of $C_{24}H_{32}O_4S$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar intensities to, those in the spectrum of *spironolactone R.S.*, Appendix 5.15 B.

(B) Complies with the test for *identification of steroids*, Appendix 3.3.11, using *solvent II* and *mobile phase D*.

(C) Shake 10 mg with 2 ml of *sulphuric acid (50 per cent v/v)*; an orange solution with intense yellowish-green fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulphide which blackens *lead acetate paper*, is evolved. Pour the solution into *water*; a greenish yellow opalescent solution is produced.

(D) It melts at about 205°, Appendix 5.11.

Specific optical rotation : Between −47.5° and −52.5°, determined in a 1.0 per cent w/v solution in *dioxan*, Appendix 5.12.

Chromium : Mix 0.20 g with 1 g of *potassium carbonate* and 0.3 g of *potassium nitrate* in a platinum crucible, heat, gently until fused, and ignite at 600°C to 650° until the carbon is removed. Cool, dissolve the residue as completely as possible in 10 ml of *water* with the aid of gentle heat, filter and add sufficient *water* to produce 20 ml; to 10 ml add 0.5 g of *urea* and just acidify with *sulphuric acid (14 per cent v/v)*. When effervescence ceases, add a further 1 ml of *sulphuric acid (14 per cent v/v)*, dilute to 20 ml with *water* and add 0.5 ml of *diphenylcarbazide solution*. The colour produced is not deeper than that produced by adding 1 ml of *sulphuric acid (14 per cent v/v)* to 0.5 ml of a freshly prepared 0.00283 per cent w/v solution of *potassium dichromate*, diluting to 20 ml with *water* and adding 0.5 ml of *diphenylcarbazide solution*.

Mercapto compounds : Shake 2.0 g with 20 ml of *water*, filter and titrate 10 ml of the filtrate with *0.01N iodine* using *starch solution* as indicator; not more than 0.1 ml of *0.01N iodine* is required.

Sulphur : Between 7.45 and 7.05 per cent, determined by the *oxygen-flask method for sulphur*, Appendix 3.3.6, using 30 mg.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 10 mg and dissolve in sufficient *methyl alcohol* to produce 100.0 ml, dilute 10.0 ml to 100.0 ml with *methyl alcohol* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 238 nm, Appendix 5.15 A. Calculate the content of $C_{24}H_{32}O_4S$, taking 470 as the value of E(1 per cent, 1-cm) at the maximum at about 238 nm.

Storage : Store in well-closed, light-resistant containers.

Starch

Category : Pharmaceutical aid (filler, binder, disintegrant); dusting powder.

Description : Fine, white powder or irregular, angular white masses, readily reducible to powder; odourless; taste, slight and characteristic.

Solubility : Practically insoluble in cold *water* and in *alcohol*.

Standards : Starch consists of polysaccharide granules obtained from the grains of maize (*Zea mays* Linn.) or rice (*Oryza sativa* Linn.) or of wheat (*Triticum aestivum* Linn.) (Fam. Gramineae), or from the tubers of the potato (*Solanum tuberosum* Linn.).

Microscopical characteristics : *Maize starch* – Consists of angular polyhedral or rounded granules upto 30 µm in size, and exhibiting in the centre a two-to-five rayed hilum.

Rice starch – Consists of simple and compound granules or more often aggregates of granules; single grains, polyhedral, about 2 to 10 µm in size, and exhibiting a minute central hilum.

Wheat starch – Consists principally of simple, large and small granules, outline circular, oval or subreniform; smaller size range from 5 to 15 µm; larger upto 50 µm in greatest diameter; hilum, appearing as a central point; striations, faintly marked, concentric; compound granules, a few, of two to four components.

Potato starch – Consists principally of simple granules, irregularly ovoid, upto 100 µm in size; hilum, near the narrower end; striations, well marked, concentric; a few compound granules of two to four components sometimes present.

Identification : (A) Heat to boiling for one minute a suspension of 1 g in 50 ml of *water* and cool. A thin and cloudy mucilage is produced with all starches except potato starch which gives a thick and more transparent mucilage.

(B) To 10 ml of mucilage obtained in **Identification**

test (A) add one drop of *0.01 N iodine*; a dark blue colour is produced.

Acidity : Add 10 g to 100 ml of *alcohol (70 per cent)* previously neutralised to *phenolphthalein solution*; shake well during one hour, filter and titrate 50 ml of the filtrate with *0.1 N sodium hydroxide*, using *phenolphthalein solution* as indicator; not more than 2 ml of *0.1 N sodium hydroxide* is required.

Iron : Dissolve the residue obtained in the test for **Sulphated ash** in 4 ml of *hydrochloric acid* with the aid of gentle heat; dilute with *water* to 50 ml and mix 25 ml comply with the *limit test for iron*, Appendix 3.5.

Microbial limits : Meets the requirements of the test for absence of *E. coli* and *Salmonella*, Appendix 4.5.

Fluorescence : No fluorescence should be visible under screened ultra-violet light.

Oxidising substance : To 5 g add 10 ml of *water* and 1 ml of *acetic acid* and stir until a homogeneous suspension is obtained. Add 0.5 ml of a freshly prepared, saturated solution of *potassium iodide*, mix, and allow to stand for five minutes. No distinct brown or blue colour is observed.

Sulphated ash : Not more than 0.6 per cent (for all starches except rice starch) and not more than 0.8 per cent (for rice starch), determined on 2.0 g, Appendix 3.2.7.

Loss on drying : Not more than 14.0 per cent (for all starches except potato starch) and not more than 20.0 per cent (for potato starch) determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Storage : Store in well-closed containers, protected from moisture.

Labelling : The label on the container states the type of starch.

Stearic Acid

Category : Pharmaceutical aid (tablet lubricant; emulsion adjunct).

Description : Hard, white or faintly yellowish, somewhat glossy, crystalline, solid or powder; odour and taste, slightly tallow-like.

Solubility : Practically insoluble in *water*, soluble in *alcohol*; freely soluble in *chloroform* and *solvent ether*.

Standards : Stearic Acid consists of a mixture of fatty acids chiefly of stearic acid, $C_{17}H_{35}COOH$, and palmitic acid, $C_{15}H_{31}COOH$.

Congealing temperature : Congeals at a temperature not below 54°, Appendix 5.5.

Iodine value : Not more than 4, Appendix 3.3.18.

Mineral acid : Shake 5 g of melted stearic acid with an equal volume of hot *water* for 2 minutes, cool, and filter; to the filtrate add one drop of *methyl orange solution*; no red colour is produced.

Neutral fat or paraffin : To 1 g add 0.5 g of *sodium carbonate* and 30 ml of *water* and boil. The resulting solution is clear or at the most opalescent while hot.

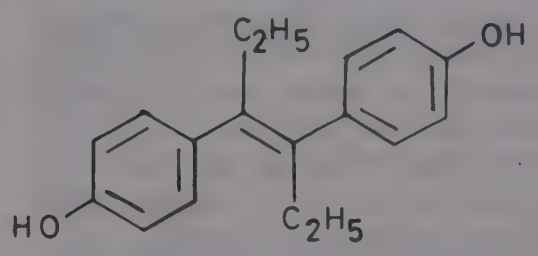
Heavy metals : Not more than 10 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Storage : Store in well-closed containers.

Stilboestrol

Diethylstilboestrol



$C_{18}H_{20}O_2$ Mol. Wt. 268.35

Category : Oestrogen.

Dose : In the treatment of menopausal symptoms, 0.1 to 1 mg daily. For the suppression of lactation, 5 mg thrice daily for three days, followed by 5 mg daily for six days.

In the treatment of carcinoma of the prostate and mammary carcinoma, 10 to 20 mg daily.

Description : White or almost white, crystalline powder, odourless.

Solubility : Very slightly soluble in *water*; freely soluble in *alcohol* and in *solvent ether*; sparingly soluble in *arachis oil*; also soluble in aqueous solutions of alkali hydroxides.

Standards : Stilboestrol is (*E*)-α, β-diethylstilbene-4,4′-diol. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{18}H_{20}O_2$, calculated with reference to the dried substance.

Identification : (A) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v

solution in *ethyl alcohol* exhibits a maximum at 241 nm; *extinction* at 241 nm, about 0.6, Appendix 5.15 A.

(B) The light absorption, in the range 230 to 450 nm, of the irradiated solution prepared as directed in the **Assay**, exhibits two maxima, at 292 nm and 418 nm, Appendix 5.15 A.

(C) It melts at about 172°, Appendix 5.11.

(D) Dissolve 0.5 mg in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid*, and heat in a water-bath for three minutes. A deep yellow colour is produced which almost disappears on dilution with 3 ml of *glacial acetic acid* (distinction from dienoestrol).

4,4'-Dihydroxystilbene and related ethers : *Extinction* of a 1-cm layer of a 1.0 per cent w/v solution in *ethyl alcohol* at 325 nm, not more than 0.5, Appendix 5.15 A.

Mono- and di-methyl ethers : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 1 volume of *diethylamine* and 9 volumes of *benzene* as the mobile phase. Apply separately to the plate 1 µl of each of two solutions in *alcohol* containing (1) 10 per cent w/v of the substance being examined and (2) 0.05 per cent w/v of *stilboestrol monomethyl ether R.S.* and of *stilboestrol dimethyl ether R.S.* After removal of the plate, allow it to dry in air, spray with a 20 per cent w/v solution of *chlorosulphonic acid* in a mixture of equal parts of *glacial acetic acid* and *solvent ether*, and heat at 100° for five minutes. The spots in the chromatogram obtained with solution (2) are more intense than any corresponding spots in the chromatogram obtained with solution (1).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g, by drying in an oven at 105°, Appendix 5.8.

Assay : Dissolve 20 mg in sufficient *ethyl alcohol* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *ethyl alcohol*. To 25.0 ml of the resulting solution add 25.0 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen phosphate* in 55 ml of *water*, transfer a portion of the mixture to a 1-cm closed quartz cell, place the cell 10 cm from a 15-watt short-wave, ultra-violet lamp, and irradiate for ten minutes. Measure the *extinction* of the irradiated solution at the maximum at about 418 nm, Appendix 5.15 A, and calculate the content of $C_{18}H_{20}O_2$ from the *extinction* obtained by repeating the operation with *stilboestrol R.S.*

Storage : Store in well-closed, light-resistant containers.

Stilboestrol Tablets

Diethylstilboestrol Tablets

Category : Oestrogen.

Dose : Stilboestrol, in the treatment of menopausal symptoms, 0.1 to 1 mg daily. For the suppression of lactation, 5 mg thrice daily for three days, followed by 5 mg daily for six days. In the treatment of carcinoma of the prostate and mammary carcinoma, 10 to 20 mg daily.

Usual strengths : 0.5 mg; 1 mg; 5 mg; 25 mg.

Standards : Stilboestrol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of stilboestrol, $C_{18}H_{20}O_2$. The tablets may be coated.

Identification : Extract a quantity of the powdered tablets equivalent to 3 mg of Stilboestrol with *solvent ether*, filter, and evaporate the filtrate to dryness. The residue complies with **Identification** tests (A), (B) and (D), described under Stilboestrol.

Uniformity of content : This test is applicable to tablets containing 5 mg or less of stilboestrol.

Finely crush one tablet, add 10 ml of *ethyl alcohol*, shake for thirty minutes, add sufficient *ethyl alcohol* to produce 25 ml and centrifuge. Pipette an aliquot of the supernatant liquid equivalent to about 0.5 mg of Stilboestrol and carry out the **Assay** described under stilboestrol, beginning at the words "add 25 ml of a solution.....".

Repeat the operation using a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 85 and 115 per cent of the average except that for one tablet the content may be between 80 and 120 per cent of the average.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 5 mg of Stilboestrol, add 5 ml of *ethyl alcohol*, shake for fifteen minutes, add sufficient *ethyl alcohol*, to produce 100.0 ml, and centrifuge. Dilute 20.0 ml of the clear supernatant liquid to 50.0 ml with *ethyl alcohol*. Using 25 ml of the resulting solution complete the **Assay** described under Stilboestrol, beginning at the words "add 25 ml of a solution.....".

Storage : Store in well-closed, light-resistant containers.

Stilboestrol Diphosphate

Diethylstilboestrol Diphosphate; Fosfertrol

$C_{18}H_{22}O_8P_2$ Mol. Wt. 428.3

Category : Oestrogen.

Dose : By intravenous infusion, 0.5 g followed by 1 g daily for five days and then 0.25 to 0.5 g once a week.

Description : White or almost white crystalline powder; odourless.

Solubility : Sparingly soluble in *water*; soluble in *alcohol* and in dilute alkalies.

Standards : Stilboestrol Diphosphate is Dihydrogen 3,4-bis (p-phosphonyl-oxyphenyl) hex-3-ene. It contains not less than 95.0 per cent and not more than the equivalent of 101.0 per cent of $C_{18}H_{22}O_8P_2$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *stilboestrol diphosphate R.S.*, Appendix 5.15 B.

(B) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *ethyl alcohol* exhibits a maximum only at 241 nm, Appendix 5.15 A.

(C) Dissolve about 15 mg in 1 ml of *sulphuric acid*; an orange colour is produced which disappears on dilution with 10 ml of *water*.

Free diethylstilboestrol : Not more than 0.15 per cent, determined in the following manner: Weigh accurately about 0.5 g, add 20 ml of *water* and sufficient *N sodium hydroxide* to bring the pH of the solution to 8.0 (determined in a pH-meter). Transfer to a separator and extract with four quantities, each of 20 ml of peroxide-free *solvent ether*. Evaporate the combined ether extract on a water-bath to about 50 ml, transfer to a glass-stoppered 50-ml conical flask and evaporate to dryness. Add 10.0 ml of *vanadyl sulphate solution* and heat the loosely-stoppered flask on a water-bath for one hour. Cool and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 520 nm against a blank consisting of 10.0 ml of *vanadyl sulphate solution*, treated in a similar manner, Appendix 5.15 A. Calculate the content of diethylstilboestrol from the *extinction* obtained by repeating the operation with *diethylstilboestrol R.S.*

Diethylstilboestrol monophosphate : Not more than 1.5 per cent, determined in the following manner: Weigh accurately about 50 mg, add 20 ml of *water* and sufficient *N sodium hydroxide* or *dilute phosphoric acid* to bring the pH of the solution to about 2.2 (determined in a pH-meter). Proceed as directed in the test for **Free diethylstilboestrol**, beginning at the words "Transfer to a separator.....". Calculate the quantity in mg of diethylstilboestrol monophosphate in the portion of Diethylstilboestrol Diphosphate taken by the formula 1.298(T−F), where T is the total quantity of free diethylstilboestrol and diethylstilboestrol diphosphate expressed as diethylstilboestrol and determined in the manner described in this test, and F is the content in mg of free diethylstilboestrol in the sample taken and determined as described in the test for free diethylstilboestrol.

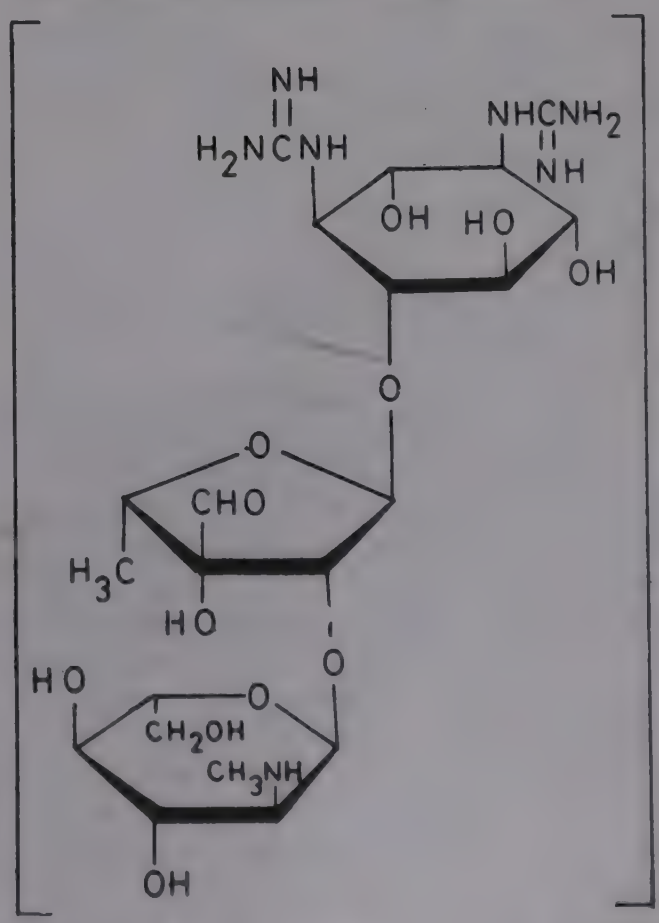
Sulphated ash : Not more than 3.0 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for four hours, Appendix 5.8.

Assay : Weigh accurately about 0.1 g, add 25 ml of *alcohol*, 5 ml of *N sodium hydroxide* and sufficient *water* to produce 250 ml. Dilute 5.0 ml to 100.0 ml with *water* and mix. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 241 nm, Appendix 5.15 A. Calculate the content of $C_{18}H_{22}O_8P_2$, from the *extinction* obtained by repeating the operation with *diethylstilboestrol diphosphate R.S.*

Storage : Store in tightly-closed containers and in a cool place.

Streptomycin Sulphate



$(C_{21}H_{39}N_7O_{12})_2, 3H_2SO_4$ Mol. Wt. 1457.38

Category : Antibacterial (tuberculostatic).

Dose : By intramuscular injection, the equivalent of 0.5 to 1.0 g of streptomycin base daily, or at longer intervals.

As an intestinal antiseptic, the equivalent of 0.5 g of streptomycin base every eight hours.

Description : White or almost white powder; odourless or with a slight odour; taste, slightly bitter; hygroscopic.

Solubility : Very soluble in *water*; slightly soluble in *alcohol*; practically insoluble in *chloroform* and in *solvent ether*.

Standards : Streptomycin Sulphate is the sulphate of *O*-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-N^3,N^3-diamidino-D-streptamine, a substance produced by the growth of certain strains of *Streptomyces griseus*, or obtained by any other means. It has a potency equivalent to not less than 700 μg and not more than 850 μg of streptomycin per mg. It contains not less than 90.0 per cent of the stated amount of streptomycin base, $C_{21}H_{39}N_7O_{12}$.

Identification : (A) Dissolve 10 mg in 5 ml of *water*, add 1 ml of *N sodium hydroxide*, and heat on a water-bath for about five minutes. Cool, and add 2 ml of a solution of 0.2 g of *ferric ammonium sulphate* in 10 ml of *N sulphuric acid*; a purple red colour is produced.

(B) To a solution of 0.5 mg in 5 ml of *water* add 1 ml of *sodium hydroxide solution* and 1 ml of a 0.05 per cent w/v solution of *α-naphthol* in *alcohol (50 per cent)*. Cool to 15° and add 3 drops of *sodium hypobromite solution*; a violet-red colour is produced.

(C) a solution (1 in 20) gives the reactions of *sulphates*, Appendix 3.1.

pH : Between 4.5 and 7.0, determined in a 25 per cent w/v solution, Appendix 5.10.

Alcohols : Not more than 3 per cent, calculated as CH_4O and determined by the following method: Weigh accurately about 0.2 g, transfer to a flask, dissolve in 5 ml of *water* and add 0.05 ml of *0.05N sulphuric acid*, connect the flask to a distillation apparatus and collect about 2.5 ml of the distillate in a 10-ml test-tube. Transfer to a conical flask, rinsing the test-tube with two quantities, each of 1 ml, of *water*, and add 25.0 ml of *0.1N potassium dichromate* containing 40 per cent v/v of *sulphuric acid*. Heat for thirty minutes on a water-bath, cool, and dilute to about 500 ml with *water*. Add 10 ml of a 10 per cent w/v solution of *potassium iodide*, allow to stand for five minutes and then titrate with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator, until the dark blue colour turns pale green. Repeat the operation using 5 ml of *water* and beginning at the words "add 0.05 ml of *0.05N sulphuric acid*....."; the difference between the titrations represents the amount of *0.1N sodium thiosulphate* equivalent to the alcohols present. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.534 mg of CH_4O.

Sulphated ash : Not more than 1.0 per cent, determined on 1.0 g, Appendix 3.2.7.

Loss on drying : Not more than 5.0 per cent, determined on 1.0 g by drying "in vacuo at 60°" for four hours, Appendix 5.8.

Assay : (1) *For potency* – Carry out the *microbiological assay of antibiotics, Method A or B*, Appendix 4.1, and express the results in μg of streptomycin per mg.

(2) *For streptomycin base* – Weigh accurately about 0.2 g and dissolve in sufficient *water* to produce 100.0 ml. To 5.0 ml add 5.0 ml of *0.2N sodium hydroxide* and heat for exactly ten minutes in a water-bath. Cool in ice for exactly five minutes, add 3 ml of a 1.5 per cent w/v solution of *ferric ammonium sulphate* in *0.5N sulphuric acid* and sufficient *water* to produce 25.0 ml and mix. Exactly twenty minutes after the addition of the ferric ammonium sulphate measure the *extinction* of a 1-cm layer of the solution at the maximum at about 525 nm, using as the blank a solution prepared in the same manner, omitting the substance being examined, Appendix 5.15 A. Calculate the content of $C_{21}H_{39}N_7O_{12}$, from the *extinction* obtained by carrying out the operations simultaneously using about 0.2 g accurately weighed, of *streptomycin sulphate R.S.* and from the declared content of $C_{21}H_{39}N_7O_{12}$ in the *streptomycin sulphate R.S.*

Streptomycin Sulphate intended for parenteral administration complies with the following additional requirements :

Histamine-like substances : Complies with the *test for histamine-like substances*, Appendix 2.35, using a solution containing 3.0 mg in 1 ml of sterile *saline solution*.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using not less than 12 mg per kg of the rabbit's weight, dissolved in not more than 5 ml of *water for injection*.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Undue toxicity : Complies with the test described under Bacitracin, using 0.5 ml of a solution containing the equivalent of 1.0 mg of streptomycin in *water for injection*.

Storage : Store in well-closed containers, protected from moisture and at a temperature not exceeding 30°. If it is intended for parenteral administration, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling : The label on the container states (1) the

equivalent weight of streptomycin base contained in it; (2) the date after which the contents are not intended to be used; (3) the storage conditions; (4) whether or not the contents are intended for parenteral administration.

Streptomycin Injection

Category : Antibacterial (tuberculostatic).

Dose : By intramuscular injection, the equivalent of 0.5 to 1 g of streptomycin base daily, or at longer intervals.

Usual strengths : The equivalent of 0.75 g and 1 g of streptomycin base.

Standards : Streptomycin Injection is a sterile solution of Streptomycin Sulphate in Water for Injection. It is prepared by dissolving the contents of a sealed container in the requisite amount of Water for Injection.

Content of streptomycin base : Determine the weight of the contents of each of ten containers as described in the test for **Uniformity of weight** under Injections. Mix the contents of the ten containers and carry out the *microbiological assay of antibiotics, Method A or B*, Appendix 4.1. From the result of the assay calculate the proportionate amount of streptomycin base in each container. This amount is not less than 90.0 per cent and not more than 115.0 per cent of the amount stated on the label.

Other requirements : Complies with the requirements stated under Injections.

The contents of the sealed container comply with the following requirements:

Description : White or almost white powder; odourless.

Identification, pH, Pyrogens, Sterility, Histamine-like substances and Undue toxicity : Comply with the requirements stated under Streptomycin Sulphate.

Loss on drying : Not more than 7.0 per cent, determined on 1.0 g by drying "in vacuo at 60°" for four hours, Appendix 5.8.

Storage : Store in a cool, dry place. The constituted solution should be used within four days when stored in a cold place.

Labelling : The label on the sealed container states (1) the quantity of Streptomycin Sulphate contained in it in terms of the equivalent amount of streptomycin base; (2) the date after which the contents are not intended to be used; (3) the storage conditions.

Streptomycin Tablets

Streptomycin Sulphate Tablets

Category : Antibacterial (intestinal antiseptic).

Dose : The equivalent of 0.5 g of streptomycin base, every eight hours.

Usual strength : The equivalent of 0.5 g of streptomycin base.

Standards : Streptomycin Tablets contain a quantity of Streptomycin Sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of streptomycin base.

Identification : (A) Triturate a quantity of the powdered tablets equivalent to 0.2 g of streptomycin in a mixture of 2 ml of *methyl alcohol* and 0.1 ml of *sulphuric acid*, filter, if necessary, and allow to stand at about 25°; crystals of streptidine sulphate separate in the course of two or three days. Dissolve the crystals in a solution of 0.1 g of *picric acid* in 10 ml of hot *water* and cool; the precipitate, after recrystallisation from hot *water*, melts at about 283° Appendix 5.11.

(B) Boil a small quantity of powdered tablets with *N sodium hydroxide* for a few minutes, add a slight excess of *hydrochloric acid* and a few drops of *ferric chloride test solution*; a brilliant violet colour is produced.

Loss on drying : Not more than 7.0 per cent, determined on 1.0 g of the powdered tablets by drying "in vacuo at 60°" for three hours, Appendix 5.8.

Other requirements : Comply with the requirements stated under Tablets.

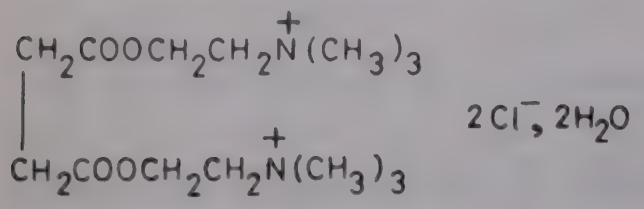
Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.3 g of streptomycin base and triturate with a few ml of *buffer solution, pH 8.0*. Dilute to 100.0 ml with *water* and carry out the *microbiological assay of antibiotics, Method A* or *B*, Appendix 4.1, and express the results in mg of streptomycin base per tablet.

Storage : Store in well-closed containers, in a cool, dry place.

Labelling : The label on the container states (1) the strength in terms of the equivalent amount of streptomycin base; (2) the date after which the tablets are not intended to be used; (3) the storage conditions.

Succinylcholine Chloride

Suxamethonium Chloride



$C_{14}H_{30}Cl_2N_2O_4, 2H_2O$ Mol. Wt. 397.34

Category : Depolarising muscular relaxant.

Dose : By intravenous injection, 20 to 80 mg.

By intravenous infusion, 1 g in 500 to 1000 ml of 5 per cent Dextrose Injection or Sodium Chloride Injection.

By intramuscular injection, upto 2.5 mg per kg of body weight, not exceeding a total dose of 150 mg.

Description : White or almost white crystalline powder; almost odourless; taste, salty.

Solubility : Freely soluble in *water*; slightly soluble in *alcohol*; practically insoluble in *solvent ether*.

Standards : Succinylcholine Chloride is the dihydrate of bis(*N*,*N*,*N*,*N'*,*N'*,*N'*-hexamethyl)-3,8-dioxa-4, 7-dioxodecamethylene-1, 10-diammonium chloride. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{14}H_{30}Cl_2N_2O_4$, calculated with reference to the anhydrous substance.

Identification : (A) Dissolve about 25 mg in 1 ml of *water* and add 0.1 ml of a 1.0 per cent w/v solution of *cobalt chloride* and 0.1 ml of *potassium ferrocyanide solution*. An emerald green colour is produced.

(B) To 0.1 g add 10 ml of *water*, 10 ml of *dilute sulphuric acid* and 30 ml of *ammonium reineckate solution*. A pink precipitate is produced. Allow to stand for 30 minutes, filter and wash with *water*, then with *alcohol* and finally with *solvent ether*. The residue after drying at 80° melts at about 180°, Appendix 5.11.

(C) A solution (1 in 20) gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 4.0 and 5.0, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Ammonium salts : To about 0.2 g add 5 ml of *sodium carbonate solution* and bring to a boil; no odour of ammonia is detected.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Water : Not more than 10.0 per cent w/w, Appendix 3.3.25.

Chloride : Between 19.3 per cent and 19.8 per cent, calculated with reference to the anhydrous substance, and determined as follows : Weigh accurately about 0.4 g and dissolve in 5 ml of *water*. Add 5 ml of *glacial acetic acid*, 50 ml of *methyl alcohol* and one drop of *eosin solution*. Titrate with *0.1N silver nitrate*. Each ml of *0.1N silver nitrate* is equivalent to 0.003545 g of Cl.

Assay : Weigh accurately about 0.5 g and dissolve in a mixture of 10 ml of *glacial acetic acid* and 10 ml of *mercuric acetate solution*. Warm slightly if necessary to effect solution. Cool, add two drops of *crystal-violet solution* and titrate with *0.1N perchloric acid*. Perform a blank titration and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.018078 g of $C_{14}H_{30}Cl_2N_2O_4$.

Storage : Store in tightly-closed, light-resistant containers.

Succinylcholine Chloride Injection

Suxamethonium Chloride Injection

Category : Depolarising muscular relaxant.

Dose : Succinylcholine Chloride. By intravenous injection, 20 to 80 mg.

By intravenous infusion, 1 g in 500 to 1000 ml of 5 per cent Dextrose Injection or Sodium Chloride Injection.

By intramuscular injection, upto 2.5 mg per kg of body weight, not exceeding a total dose of 150 mg.

Usual strength : 50 mg in 1 ml.

Standards : Succinylcholine Chloride Injection is a sterile solution of Succinylcholine Chloride in Water for Injection. It contains not less than 90.0 per cent and not more than 107.5 per cent of the stated amount of $C_{14}H_{30}Cl_2N_2O_4,2H_2O$.

Identification : (A) Dilute a volume equivalent to 20 mg of Succinylcholine Chloride to 50 ml with *water*. To 0.5 ml add 2 ml of *chloroform*, 2 ml of a solution containing 0.16 per cent w/v of *citric acid* and 6.6 per cent w/v of *sodium phosphate*, and 0.1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate*. Shake for two minutes and allow to separate; the chloroform layer is coloured yellow.

(B) Dilute a volume equivalent to 20 mg of Succinylcholine Chloride to 1 ml; the solution complies with **Identification** test (A) described under Succinylcholine Chloride.

(C) It gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 3.0 and 5.0, Appendix 5.10.

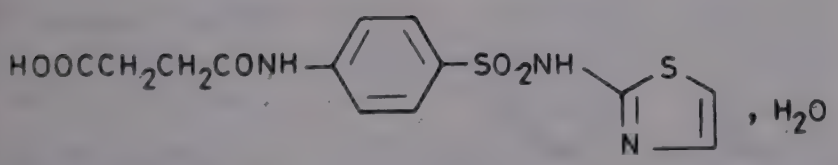
Hydrolysis products : The volume of *0.1N sodium hydroxide* required for the preliminary neutralisation in the Assay is not more than one-tenth of the total volume of *0.1N sodium hydroxide* required for the preliminary neutralisation and the hydrolysis..

Assay : To a volume equivalent to 0.2 g of Succinylcholine Chloride add 30 ml of *carbon dioxide-free water* and shake with five quantities, each of 20 ml, of *solvent ether*. Wash the combined ether extracts with two quantities, each of 10 ml, of *water* and discard the ether. Shake the combined washings with two quantities, each of 10 ml, of *solvent ether*, add the washings to the original aqueous solution and neutralise with *0.1N sodium hydroxide* using *bromothymol blue solution* as indicator. Add 25.0 ml of *0.1N sodium hydroxide*, heat under a reflux condenser for forty minutes, allow to cool, and titrate the excess of alkali with *0.1N hydrochloric acid*, using *bromothymol blue solution* as indicator. Repeat the operation using 40 ml of *carbon dioxide-free water* and beginning at the words "add 25.0 ml....", the difference between the titrations represents the volume of *0.1N sodium hydroxide* required for the hydrolysis of succinylcholine chloride. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01987 g of $C_{14}H_{30}Cl_2N_2O_4, 2H_2O$.

Storage : Store in single-dose or multiple-dose containers at a temperature not exceeding 4°

Labelling : The label on the container states (1) the date after which the injection is not intended to be used; (2) the storage conditions.

Succinylsulphathiazole



$C_{13}H_{13}N_3O_5S_2, H_2O$ Mol. Wt. 373.40

Category : Antibacterial.

Dose : 10 to 20 g daily, in divided doses.

Description : White or yellowish-white crystals or powder; odourless; taste, slightly bitter.

Solubility : Very slightly soluble in *water*; sparingly soluble in *alcohol*; practically insoluble in *chloroform*; soluble in solutions of alkali hydroxides, and in solution of *sodium bicarbonate* with the evolution of carbon dioxide.

Standards : Succinylsulphathiazole is the monohydrate of 4-(*N*-thiazol-2-ylsulphamoyl)succinanilic acid. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{13}H_{13}N_3O_5S_2$, calculated with reference to the dried substance.

Identification : (A) Heat about 0.1 g with 5 ml of *N sodium hydroxide* on a water-bath at 95° to 100° for one hour. Cool, dilute to 10 ml and neutralise with *dilute hydrochloric acid*; a precipitate of plate-like crystals forms. Filter, wash the precipitate with *water*, and dry at 105°; melting-range of the residue, 198° to 203°, Appendix 5.11.

(B) Boil 0.1 g with 10 ml of *dilute hydrochloric acid* for fifteen minutes; cool 2 ml of the solution in ice; add 4 ml of *sodium nitrite solution*, cool again, and pour the mixture into 2 ml of *β-naphthol solution* containing 1 g of *sodium acetate*; an orange precipitate is produced.

(C) Heat 0.5 g with 10 ml of *dilute hydrochloric acid* on a water-bath until it dissolves and evaporates to dryness. To the residue add 5 ml of *dilute ammonia solution*, evaporate to dryness and maintain at 100° for thirty minutes. Mix the residue with 2.5 g of *zinc powder*, transfer the mixture to a test-tube and heat gently over an open flame whilst holding in the mouth of the tube a strip of filter paper freshly moistened with a 5 per cent w/v solution of *dimethylaminobenzaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 20 volumes of *alcohol*. The paper turns yellow.

(D) It melts at about 190° with decomposition, Appendix 5.11.

Clarity and colour of solution : Dissolve 1.0 g in a mixture of 5 ml of *sodium hydroxide solution* and 15 ml of *water*. The solution is not more than slightly opalescent, and not more intensely coloured than a mixture of 0.24 ml of *ferric chloride C.S.*, 0.06 ml of *cobalt chloride C.S.* and 19.7 ml of *dilute hydrochloric acid* (containing 1 per cent w/v HCl).

Acidity : Heat 2.0 g with 50 ml of *water* at 70° for five minutes. Cool rapidly and filter. Titrate 25 ml with *0.1N sodium hydroxide* using *bromothymol blue solution* as indicator. Not more than 1.0 ml of *0.1N sodium hydroxide* is required.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined in the following manner: Dissolve 0.5 g in a mixture of 5 ml of *sodium hydroxide solution* and 20 ml of *water* and add five drops of *sodium sulphide solution*. The colour of the solution is not more intense than that of a solution containing 5 ml of *sodium hydroxide solution*, 20 ml of *water* and 1.0 ml of *standard lead solution*.

Chloride : Heat 2 g with 50 ml of *water* at about 70° for

five minutes, cool and filter. 25 ml of the filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : Heat 2 g with 50 ml of *water* at about 70° for five minutes, cool and filter. 25 ml of the filtrate complies with the *limit test for sulphates*, Appendix 3.2.8.

Sulphathiazole : Dissolve 20 mg in 25 ml of *alcohol*, add 3.5 ml of *water* and 6 ml of *dilute hydrochloric acid*, cool, add 1 ml of a 0.25 per cent w/v solution of *sodium nitrite* and allow to stand for three minutes. Add 2.5 ml of a 4 per cent w/v solution of *sulphamic acid* and allow to stand for five minutes. Add 1 ml of a 0.4 per cent w/v solution of *N-(1-naphthyl) ethylenediamine hydrochloride* and dilute to 50 ml with *water*. The solution is not more intensely coloured than a solution prepared simultaneously, consisting of a mixture of 25 ml of *alcohol*, 2 ml of *water*, 6 ml of *dilute hydrochloric acid* and 1.5 ml of solution containing in 100 ml, 10 mg of *sulphathiazole* and 0.5 ml of *hydrochloric acid* and treated in the same manner.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not less than 4.0 per cent and not more than 5.5 per cent determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and dissolve in 100 ml of a mixture of 1 volume of *hydrochloric acid* and 2 volumes of *water*. Boil for one hour under a reflux condenser. Cool and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03554 g of $C_{13}H_{13}N_3O_5S_2$.

Storage : Store in well-closed, light-resistant containers.

Succinylsulphathiazole Tablets

Category : Antibacterial.

Dose : Succinylsulphathiazole, 10 to 20 g daily, in divided doses.

Usual strength : 0.5 g.

Standards : Succinylsulphathiazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of succinylsulphathiazole, $C_{13}H_{13}N_3O_5S_2,H_2O$.

Identification : (A) Extract a quantity of the powdered tablets equivalent to 0.1 g of Succinylsulphathiazole with hot *acetone* and evaporate the extract to dryness. The residue, after drying at 105°, complies with the following: To 10 mg add 20 mg of *phenol* and three drops of *sulphuric acid*, heat until the solution becomes brown, cool, add 20 ml of *water*, and make alkaline with *sodium hydroxide solution*; no colour is produced (distinction from phthalylsulphathiazole).

(B) Heat a quantity of the powdered tablets equivalent to 0.3 g of Succinylsulphathiazole with 10 ml of *sodium hydroxide solution* in a water-bath for one hour. Cool, add 15 ml of *water* and filter, and neutralise the filtrate with *dilute hydrochloric acid*. The precipitate, after washing with *water* and drying at 105°, melts at about 200°, Appendix 5.11, and complies with **Identification** test (B) described under Succinylsulphathiazole.

Sulphathiazole : Dissolve a quantity of the powdered tablets equivalent to 5.0 mg of Succinylsulphathiazole as completely as possible in a cooled mixture of 25 ml of *alcohol*, 12.5 ml of *N hydrochloric acid* and 7 ml of *water*, and filter. To the filtrate add 1 ml of a 0.25 per cent w/v solution of *sodium nitrite*, mix, allow to stand for three minutes and complete the test described under Succinylsulphathiazole, beginning at the words "add 2.5 ml of a 4 per cent w/v solution. . . .".

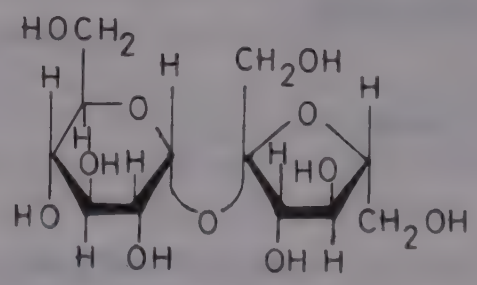
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.8 g of Succinylsulphathiazole and carry out the **Assay** described under Phthalylsulphathiazole Tablets. Each ml of *0.1M sodium nitrite* is equivalent to 0.03734 g of $C_{13}H_{13}N_3O_5S_2,H_2O$.

Storage : Store in well-closed, light-resistant containers.

Sucrose

Refined Sugar



$C_{12}H_{22}O_{11}$ Mol. Wt. 342.30

Category : Pharmaceutical aid (sweetening agent; tablet excipient).

Description : Colourless crystals or crystalline masses or nearly white crystalline powder; odourless; taste, sweet.

Solubility : Very soluble in *water*; freely soluble in *alcohol (70 per cent)*; sparingly soluble in *ethyl*

alcohol; insoluble in *chloroform* and in *solvent ether*.

Standards : Sucrose is β-D-fructofuranosyl-α-D-glucopyranoside and is a sugar obtained from the juice of sugar-cane or of the sugar-beet. It contains no added substances.

Identification : (A) When heated, it melts, swells up and burns, giving off an odour of burnt sugar, and leaving a bulky carbonaceous residue.

(B) Hydrolyse a solution in *water* by boiling with *0.1N sulphuric acid* and neutralise with *sodium hydroxide solution*. Add *potassium cupri-tartrate solution* and heat; a copious red precipitate is produced.

Specific optical rotation : Not less than +65.9°, determined in a 20.0 per cent w/v solution, Appendix 5.12.

Acidity or Alkalinity : Dissolve 5.0 g in sufficient *water* to produce 10 ml. Add three drops of *phenolphthalein solution*. The solution is colourless. Titrate with *0.01N sodium hydroxide* to a pink colour. Not more than 0.25 ml of *0.01N sodium hydroxide* is required.

Calcium : To 10 ml of a 10.0 per cent w/v solution add 1 ml of *ammonium oxalate solution*; the solution remains clear for at least one minute.

Barium : Acidify 10 ml of a 10.0 per cent w/v solution with *dilute sulphuric acid* and allow to stand for twenty-four hours; no turbidity is produced.

Heavy metals : Not more than 5 parts per million, determined by method A on a solution prepared by dissolving 4.0 g in 15 ml of *water*; 0.1 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml, Appendix 3.2.4.

Sulphites : Dissolve 2.0 g in 20 ml of *water* without heating, add 0.05 ml of *0.1N iodine* and one drop of *starch solution*. A blue colour develops.

Dextrine : Dissolve 1.0 g in 10 ml of *water* and add one drop of *dilute hydrochloric acid* and one drop of *0.1N iodine*; the solution remains yellow.

Reducing sugars : Dissolve 10.0 g in 20 ml of *water*, add 5 ml of *potassium cupri-tartrate solution*, boil for five minutes and cool. The solution remains blue and clear and does not form an orange coloured precipitate within one hour.

Foreign colouring matter : (A) Dissolve 50 g in sufficient *water* to produce 100 ml and add 1 ml of *dilute hypophosphorous acid*. No unpleasant odour in given off for at least one hour.

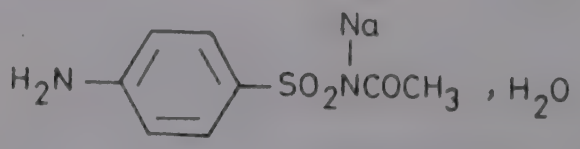
(B) Examine the substance under ultra-violet light at 365 nm; no fluorescence is noticed.

sulphated ash : Not more than 0.02 per cent, Appendix 3.2.7.

Storage : Store in well-closed containers.

Sulphacetamide Sodium

Soluble Sulphacetamide



$C_8H_9N_2NaO_3S,H_2O$ Mol. Wt. 254.24

Category : Antibacterial (ophthalmic).

Description : White or yellowish-white crystals or microcrystalline powder; odourless; taste, slightly bitter.

Solubility : Freely soluble in *water*; sparingly soluble in *alcohol*; practically insoluble in *benzene*, in *chloroform* and in *solvent ether*.

Standards : Sulphacetamide Sodium is the monohydrate of the sodium salt of N^1- acetylsulphanilamide

It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_8H_9N_2NaO_3S$, calculated with reference to the anhydrous substance.

Identification : (A) Dissolve 1 g in 10 ml of *water* and add 2 ml of *acetic acid*; a white precipitate is produced. Collect the precipitate, wash with cold *water*, and dry at 105° for four hours. The residue melts at about 183° and complies with **Identification** test (B) described under Succinylsulphathiazole.

(B) Heat the residue obtained in **Identification** test (A) with *alcohol* and *sulphuric acid*; ethyl acetate, recognisable by its odour, is produced.

(C) Incinerate 0.5 g; the residue gives the reactions of *sodium*, Appendix 3.1.

pH : Between 8.0 and 9.5, determined in a 5.0 per cent w/v solution, Appendix 5.10.

Clarity and colour of solution : A solution of 1.0 g in a mixture of 1 ml of *sodium hydroxide solution* and 19 ml of *water* is clear and the colour of the solution is not more intense than that of a mixture of 0.6 ml of *ferric chloride C.S.*, 0.15 ml of *cobalt chloride C.S.*, 0.15 ml of *copper sulphate C.S.*, and sufficient *water* to produce 20.0 ml.

Heavy metals : Not more than 20 parts per million, determined in the following manner: Dissolve 1 g in 25 ml of *water* and add five drops of *sodium sulphide solution*; any colour produced is not darker than that of a solution containing 25 ml of *water*, 2 ml of *standard lead solution* and five drops of *sodium sulphide solution*.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel GF 254* precoated plate and a mixture of 50 volumes of *n-butyl alcohol*, 25 volumes of *ethyl alcohol*, 25 volumes of *water* and 10 volumes of *strong ammonia solution* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions containing (1) 10.0 per cent w/v of the substance being examined, (2) 0.050 per cent w/v of *sulphanilamide*, and (3) 0.025 per cent w/v of *sulphanilamide*. After removal of the plate, allow it to dry in air and spray with a 2 per cent w/v solution, prepared without heating, of *4-dimethylaminobenzaldehyde* in a 55 per cent w/v solution of *hydrochloric acid*. Any spot in the chromatogram obtained with solution (1), other than the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) and not more than one such spot may be more intense than the spot in the chromatogram obtained with solution (3).

Loss on drying : Between 6.0 per cent and 8.0 per cent w/w, Appendix 5.8.

Assay : Weigh accurately about 0.5 g, dissolve in a mixture of 75 ml of *water* and 10 ml of *hydrochloric acid* and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02362 g of $C_8H_9N_2NaO_3S$.

Storage : Store in tightly-closed, light-resistant containers.

Sulphacetamide Eye Ointment

Category : Antibacterial (ophthalmic).

Usual strengths : 5.0 per cent and 10 per cent.

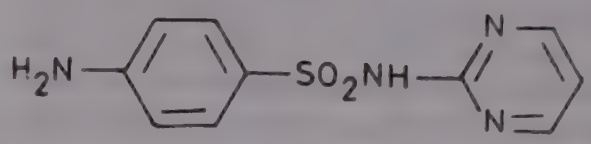
Standards : Sulphacetamide Eye Ointment contains not less than 94.0 per cent and not more than 106.0 per cent of the stated amount of Sulphacetamide Sodium, $C_8H_9N_2NaO_3S,H_2O$ in a suitable eye ointment base.

Identification : Dissolve a quantity equivalent to about 1 g of Sulphacetamide Sodium in 100 ml of *solvent ether* in a separator, and extract the mixture with 25 ml of *water*. Wash the extract with 25 ml of *solvent ether*, and warm the water extract on a water-bath to remove the last traces of ether. Adjust the pH to between 4 and 5 with *acetic acid*, and filter. Wash the precipitate with *water* and dry at 105° for two hours; the sulphacetamide so obtained has a melting range between 180° to 184°, Appendix 5.11, and complies with the **Identification** tests (A) to (C) described under Sulphacetamide.

Other requirements : Complies with the requirements stated under Eye Ointments.

Assay : Weigh accurately a quantity equivalent to 0.25 g of Sulphacetamide Sodium and transfer to a separator with the aid of 20 ml of *light petroleum (boiling range, 40° to 60°)*, 30 ml of *solvent ether*, and two quantities, each of 5 ml of *dilute hydrochloric acid*. Shake well, allow to separate, remove the acid layer, and repeat the extraction with two further quantities, each of 30 ml, of *dilute hydrochloric acid*. On the combined acid extracts carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02542 g of $C_8H_9N_2NaO_3S,H_2O$.

Sulphadiazine



$C_{10}H_{10}N_4O_2S$ Mol. Wt. 250.27

Category : Antibacterial.

Dose : Initial dose 3 g; subsequent doses, upto 4 g daily, in divided doses.

Description : White or yellowish-white or pinkish white crystals or crystalline powder; almost odourless; tasteless.

Solubility : Practically insoluble in *water*; sparingly soluble in *alcohol*, and in *acetone*; freely soluble in dilute mineral acids and in aqueous solutions of alkali hydroxides.

Standards : Sulphadiazine is N^1-(pyrimidin-2-yl) sulphanilamide. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{10}H_{10}N_4O_2S$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** test (B) described under Succinylsulphathiazole.

(B) Heat about 50 mg in a dry tube until it melts; a reddish-brown colour is produced, and the fumes which are evolved do not discolour moistened *lead acetate paper* (distinction from succinylsulphathiazole).

(C) Dissolve 10 mg in a mixture of 10 ml of *water* and 2 ml of *0.1N sodium hydroxide* and add 0.5 ml of *copper sulphate solution*; an olive-green precipitate, which becomes purple-grey on standing, is produced (distinction from certain sulphonamides).

(D) Gently heat about 1 g in a small test-tube over a small flame until a sublimate is formed; collect a few mg of

the sublimate with a glass rod and mix it in a test-tube with 1 ml of a 5 per cent w/v solution of *resorcinol* in *alcohol*. Add 1 ml of *sulphuric acid* and mix by shaking; a deep red colour appears at once. Cautiously dilute the mixture with 25 ml of ice-cold *water* and add an excess of *dilute ammonia solution*; a blue or reddish-blue colour is produced.

(E) It melts at about 255° with decomposition, Appendix 5.11.

Clarity and colour of solution : A solution of 1.0 g in a mixture of 10 ml of *N sodium hydroxide* and 15 ml of *water* is clear and the colour is not more intense than that of 25 ml of mixture of 1.45 ml of *ferric chloride C.S.*, 0.03 ml of *cobalt chloride C.S.*, 0.03 ml of *copper sulphate C.S.* and sufficient *water* to produce 100.0 ml.

Acidity : Heat 1.0 g with 50 ml of *carbon dioxide-free water* at about 70° for five minutes, cool quickly to 20° and filter; titrate 25 ml of the filtrate to pH 7.0 with *0.1N sodium hydroxide*; not more than 0.1 ml of *0.1N sodium hydroxide* is required.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Chloride : 1 g dissolved by warming in 5 ml of *nitric acid* and 5 ml of *water*, complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 1 g dissolved by warming in 5 ml of *hydrochloric acid* and 5 ml of *water*, complies with the *limit test for sulphates*, Appendix 3.2.8.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the **Assay** described under Succinylsulphathiazole. Each ml of *0.1M sodium nitrite* is equivalent to 0.02503 g of $C_{10}H_{10}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphadiazine Tablets

Category : Antibacterial.

Dose : Sulphadiazine, initial dose, 3 g; subsequent doses upto 4 g daily, in divided doses.

Usual strength : 0.5 g.

Standards : Sulphadiazine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sulphadiazine, $C_{10}H_{10}N_4O_2S$.

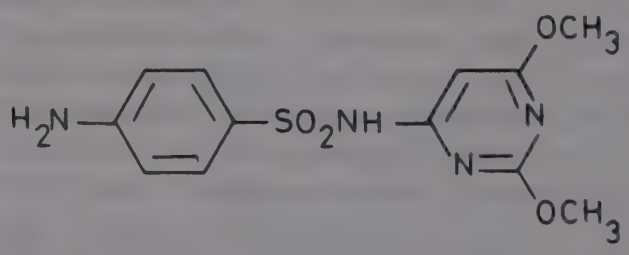
Identification : (A) Triturate a quantity of the powdered tablets, equivalent to about 0.5 g of Sulphadiazine, with two successive quantities of 5 ml of *chloroform* and reject the chloroform. Triturate the residue with 10 ml of *dilute ammonia solution* for five minutes, add 10 ml of *water* and filter. Warm the filtrate until most of the ammonia is expelled, cool, and acidify with *acetic acid*. Collect the precipitate, wash with *water*, and dry at about 100°; the residue has a melting range between 252° and 256°; Appendix 5.11 and complies with **Identification** tests (A) to (D) described under Sulphadiazine.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Sulphadiazine and dissolve as completely as possible in a mixture of 50 ml of *water* and 10 ml of *hydrochloric acid*. Carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02503 g of $C_{10}H_{10}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphadimethoxine



$C_{12}H_{14}N_4O_4S$ Mol. Wt. 310.33

Category : Antibacterial.

Dose : Initial dose, 1 to 2 g; subsequent doses, 500 mg daily.

Description : White or creamy-white, crystalline powder; almost odourless; tasteless.

Solubility : Very slightly soluble in *water*; slightly soluble in *alcohol*; soluble in dilute mineral acids and in solutions of alkali hydroxides and carbonates.

Standards : Sulphadimethoxine is N^1-(2,6-dimethoxypyrimidin-4-yl) sulphanilamide. It contains not less than 99.0 per cent of $C_{12}H_{14}N_4O_4S$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths

as, and have similar relative intensities to, those in the spectrum of *sulphadimethoxine R.S.*, Appendix 5.15 B.

(B) Dissolve about 0.1 g in 3 ml of *sodium hydroxide solution* and 50 ml of *water* and dilute to 100 ml with *water*. To 5 ml add 100 mg of *phenol*, and heat to boiling. Cool, add 0.5 ml of *sodium hypochlorite solution* and a few drops of *sodium hydroxide solution*; a yellow colour is produced.

(C) It gives the reactions of *primary aromatic amines*, Appendix 3.1, giving an orange-red precipitate

(D) Suspend about 20 mg in 5 ml of *water* and add *sodium hydroxide solution* until completely dissolved. Add a few drops of *copper sulphate solution*; the solution turns yellow, and a yellow precipitate is formed.

Melting range : Between 197° and 204°, Appendix 5.11.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel H* as the coating substance and a mixture of 20 volumes of *chloroform*, 2 volumes of *methyl alcohol* and 1 volume of *dimethylformamide*, as the mobile phase. Apply separately to the plate 10 µl of each two solutions in a mixture of 9 volumes of *alcohol* and 1 volume of *strong ammonia solution* containing (1) 0.25 per cent w/v of the substance being examined and (2) 0.00125 per cent w/v of *sulphanilamide R.S.* After removal of the plate, allow it to dry in air and spray with a 10 per cent v/v solution of *sulphuric acid* in *alcohol*, heat at 105° for 30 minutes, and immediately expose to nitrous fumes in a closed glass tank for 15 minutes [nitrous fumes may be generated by adding *sulphuric acid (50 per cent w/w)* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*]. Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl) ethylenediamine hydrochloride* in *alcohol*. If necessary, allow to dry and repeat the spraying. Any spot in the chromatogram obtained with solution (1), other than the principal spot, is not more intense than the spot in the chromatogram obtained with solution (2).

Heavy metals : Not more than 20 parts per million, determined by Method A on a solution prepared by dissolving 1 g in 5 ml of *sodium hydroxide solution* and 20 ml of *water*, Appendix 3.2.4.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Assay : Weigh accurately about 0.5 g and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03103 g of $C_{12}H_{14}N_4O_4S$.

Storage : Store in well-closed, light-resistant containers.

Sulphadimethoxine Tablets

Category : Antibacterial.

Dose : Sulphadimethoxine; initial dose, 1 to 2 g; subsequent doses 500 mg daily.

Usual strength : 500 mg.

Standards : Sulphadimethoxine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sulphadimethoxine, $C_{12}H_{14}N_4O_4S$.

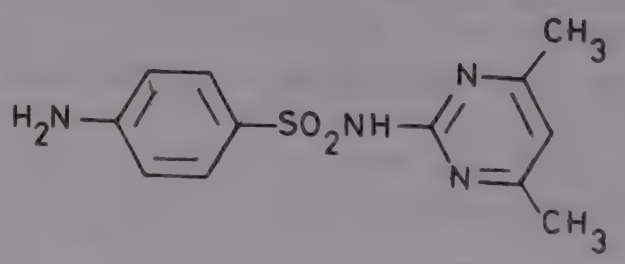
Identification : Triturate a quantity of the powdered tablets equivalent to 0.5 g of Sulphadimethoxine with 5 ml of *0.5N hydrochloric acid*; filter, and neutralise the filtrate to *litmus paper* with *0.5N sodium hydroxide*. The precipitate after washing with *water* and drying at 105°, melts at about 201°, Appendix 5.11, and complies with the **Identification** tests described under Sulphadimethoxine.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Sulphadimethoxine and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03103 g of $C_{12}H_{14}N_4O_4S$.

Storage : Store in light-resistant containers.

Sulphadimidine



$C_{12}H_{14}N_4O_2S$ Mol. Wt. 278.32

Category : Antibacterial.

Dose : In the treatment of systemic infections; initial dose, 3 g; subsequently upto 6 g daily, in divided doses.

In the treatment of urinary tract infection; initial dose, 2 g; subsequently upto 4 g in divided doses.

Description : White or creamy-white crystals or crystalline powder; almost odourless; taste, bitter.

Solubility : Very slightly soluble in *water*; soluble in *acetone*, in mineral acids, in alkalis and in alkaline

carbonates; slightly soluble in *alcohol*; practically insoluble in *solvent ether*.

Standards : Sulphadimidine is N^1-(4,6-dimethylpyrimidin-2-yl)sulphanilamide. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{12}H_{14}N_4O_2S$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** test (B) described under Succinylsulphathiazole.

(B) Dissolve about 10 mg in a mixture of 10 ml of *water* and 2 ml of *0.1N sodium hydroxide* and add 0.5 ml of *copper sulphate solution*; a pale-green turbidity appears at the first but it gradually changes to a brownish-grey suspension and finally settles as a reddish-brown precipitate (distinction from sulphacetamide).

(C) Place about 0.5 g in a test-tube. Wrap a wet filter paper around the upper portion of the tube and heat the lower portion in an oil-bath between 230° and 250°; a white, crystalline sublimate collects on the cool, upper portion of the tube. The fumes evolved during the decomposition possess a slight odour of hydrogen sulphide. Transfer the sublimate to another test-tube, sublime as previously directed, and dry over *phosphorus pentoxide* for twenty hours. The sublimate has a melting-range of 151° to 153°, a sealed capillary tube being used to contain the specimen to be melted, Appendix 5.11.

Melting range : Between 197° and 200°, Appendix 5.11.

Clarity and colour of solution : A solution of 1.0 g in 25 ml of a mixture of equal volumes of *sodium hydroxide solution* and *water* is clear and the colour is not more intense than that of a mixture of 0.3 ml of *ferric chloride C.S.*, 0.075 ml of *cobalt chloride C.S.*, and sufficient *water* to produce 25.0 ml.

Acidity : Heat 1 g with 50 ml of *carbon dioxide-free water* at about 70° for five minutes, cool quickly to 20° and filter; titrate 25 ml of the filtrate with *0.1N sodium hydroxide* using *bromothymol blue solution* as indicator; not more than 0.2 ml of *0.1N sodium hydroxide* is required.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Chloride : 1 g dissolved by warming in 5 ml of *nitric acid* and 5 ml of *water*, complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 1 g dissolved by warming in 5 ml of *hydrochloric acid* and 5 ml of *water*, complies with the *limit test for sulphates*, Appendix 3.2.8.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the **Assay** described under Succinylsulphathiazole. Each ml of *0.1M sodium nitrite* is equivalent to 0.02783 g of $C_{12}H_{14}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphadimidine Tablets

Category : Antibacterial.

Dose : Sulphadimidine. In the treatment of systemic infections, initial dose 3 g; subsequently upto 6 g daily, in divided doses. In the treatment of infections of the urinary tract: initial dose 2 g; subsequently upto 4 g daily, in divided doses.

Usual strength : 0.5 g.

Standards : Sulphadimidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sulphadimidine, $C_{12}H_{14}N_4O_2S$.

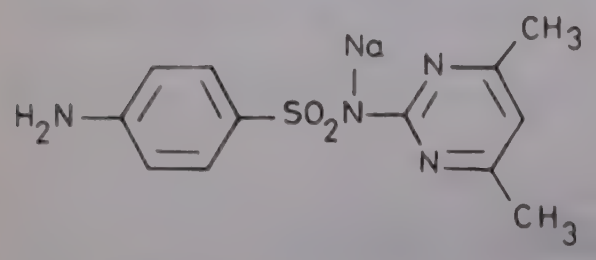
Identification : (A) Triturate a quantity of the powdered tablets, equivalent to about 0.5 g of Sulphadimidine, with two successive quantities, each of 5 ml of *chloroform*; reject the chloroform. Triturate the residue with 10 ml of *dilute ammonia solution* for five minutes, add 10 ml of *water*, and filter. Warm the filtrate until most of the ammonia is expelled, cool, and acidify with *acetic acid*. Collect the precipitate, wash with *water*, and dry at 105°; the residue has a melting range between 196° and 199°, Appendix 5.11 and complies with the **Identification** tests (A) to (C) described under Sulphadimidine.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Sulphadimidine and dissolve as completely as possible in a mixture of 50 ml of *water* and 10 ml of *hydrochloric acid*. Carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02783 g of $C_{12}H_{14}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphadimidine Sodium



$C_{12}H_{13}N_4NaO_2S$ Mol. Wt. 300.30

Category : Antibacterial.

Dose : By intramuscular or intravenous injection, 1 to 2 g.

Description : White or creamy-white crystals or crystalline powder; almost odourless; taste, bitter and alkaline; hygroscopic.

Solubility : Freely soluble in *water*; sparingly soluble in *alcohol*.

Standards : Sulphadimidine Sodium is sodium salt of N^1 -(4, 6-dimethylpyrimidine - 2 - yl) sulphanilamide. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{12}H_{13}N_4NaO_2S$, calculated with reference to the dried substance.

Identification : (A) Acidify a solution with *acetic acid*; a precipitate is produced, which, after washing with cold *water*, melts at about 198°, Appendix 5.11, and complies with **Identification** test (B) described under Succinylsulphathiazole.

(B) Incinerate 0.5 g, the residue gives the reactions of *sodium*, Appendix 3.1.

Clarity and colour of solution : Dissolve 6.66 g in sufficient *water* to produce 20.0 ml. The resulting solution is clear and the colour is not more intense than that of a mixture of 1.2 ml of *ferric chloride C.S.*, 0.3 ml of *cobalt chloride C.S.* and sufficient *water* to produce 20.0 ml.

pH : Between 10.0 and 11.0, determined in a 10 per cent w/v solution, Appendix 5.10.

Related substances : Complies with the test described under Sulphafurazole; prepare solution (1) by dissolving the substance being examined in *strong ammonia solution* and then diluting with *alcohol*.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 3.2.4.

Chloride : 1 g dissolved in 5 ml of *nitric acid* and 20 ml of *water*, complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 1 g dissolved in 5 ml of *hydrochloric acid* and 5 ml of *water*, complies with the *limit test for sulphates*, Appendix 3.2.8.

Loss on drying : Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and dissolve in a mixture of 75 ml of *water* and 10 ml of *hydrochloric acid*. Carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03003 g of $C_{12}H_{13}N_4NaO_2S$.

Storage : Store in tightly-closed, light-resistant containers.

Sulphadimidine Injection

Category : Antibacterial.

Dose : Sulphadimidine Sodium, by intramuscular or intravenous injecton, 1 to 2 g.

Usual strength : 1 g in 3 ml.

Standards : Sulphadimidine Injection is a sterile solution of Sulphadimidine Sodium in Water for Injection which is free from carbon dioxide. It contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_{12}H_{13}N_4NaO_2S$.

Identification : (A) Acidify with *acetic acid* and filter. The residue, after washing with *water* and drying at 105°, Appendix 5.11, and complies with **Identification** test (B) described under Succinylsulphathiazole.

(B) Evaporate to dryness the filtrate obtained in **Identification** test (A); the residue, after incineration, gives the reactions of *sodium*, Appendix 3.1.

Colour of solution : An injection containing 1 g in 3 ml is not more intensely coloured than a mixture of 1.2 ml of *ferric chloride C.S.*, 0.3 ml of *cobalt chloride C.S.* and 18.5 ml of *water*.

pH : Between 10.0 and 11.0, Appendix 5.10.

Related substances : Complies with the test for **Related substances** described under Sulphafurazole, using as solution (1) the injection being examined, diluted, if necessary, with *water* to contain the equivalent of 0.2 per cent w/v of sulphadimidine solution and as solution (2) a 0.002 per cent w/v solution of *sulphanilamide* in a mixture of 9 volumes of *alcohol* and 1 volume of *strong ammonia solution*.

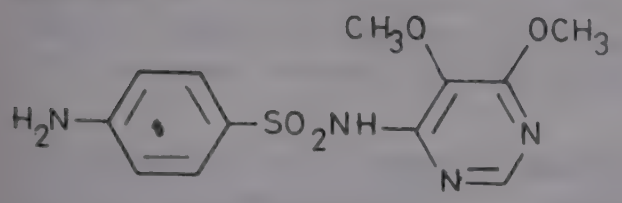
Other requirements : Complies with the requirements stated under Injections.

Assay : Carry out the **Assay** described under Sulphadimidine Sodium, using a volume equivalent to 0.5 g of Sulphadimidine Sodium and passing air slowly through

the acidified solution before titration until the odour of sulphur dioxide is no longer discernible and the vapours do not turn moistened *starch-iodate paper* blue.

Storage : Store in single-dose, light-resistant containers.

Sulphadoxine



Sulphormethoxine; Sulphorthomidine

$C_{12}H_{14}N_4O_4S$ Mol. Wt. 310.32

Category : Antibacterial.

Dose : Initial, 2 g by mouth; subsequent doses, 1 to 1.5 g weekly.

By deep intramuscular or slow intravenous injection 2.5 g initially, followed by 1.5 g after four days.

Description : White or yellowish-white, crystalline powder; odourless or almost odourless; taste, slightly bitter.

Solubility : Very slightly soluble in *water*; slightly soluble in *methyl alcohol* and in *alcohol*; soluble in dilute mineral acids and in solutions of alkali hydroxides and carbonates.

Standards : Sulphadoxine is N^1-(5,6-dimethoxy-4-pyrimidinyl) sulphanilamide. It contains not less than 99.0 per cent of $C_{12}H_{14}N_4O_4S$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *sulphadoxine R.S.*, Appendix 5.15 B.

(B) It gives the reactions of *primary aromatic amines*, Appendix 3.1, giving an orange-red precipitate.

(C) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *0.1N methanolic hydrochloric acid* exhibits a maximum only at 267 nm; *extinction* at 267 nm, about 0.56, Appendix 5.15 A.

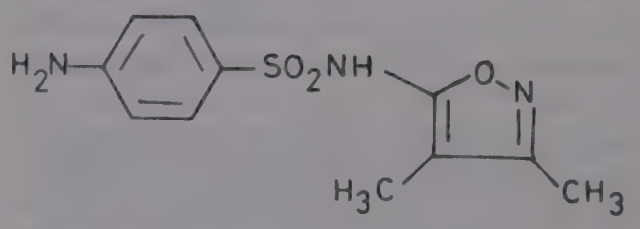
Melting range : Between 197° and 200°, Appendix 5.11.

Acidity : Heat 1.0 g with 50 ml of *carbon dioxide-free water* at about 70° for five minutes, cool quickly to 20°, and filter; 25 ml of the filtrate requires for titration to pH 7.0, not more than 0.1 ml of *0.1N sodium hydroxide*.

Heavy metals; Related substances; Sulphated ash; Loss on drying; and Assay : Complies with the requirements stated under Sulphadimethoxine.

Storage : Store in well-closed, light-resistant containers.

Sulphafurazole



$C_{11}H_{13}N_3O_3S$ Mol. Wt. 267.30

Category : Antibacterial.

Dose : In the treatment of systemic infection, initial dose, 3 g; subsequent doses, upto 6 g daily, in divided doses. In the treatment of infections of urinary tract; initial dose 2 g, subsequent doses, upto 4 g daily, in divided doses.

Description : White or yellowish-white, crystalline powder; odourless; taste, slight, becoming bitter.

Solubility : Very slightly soluble in *water*; sparingly soluble in *alcohol*, and in 5 per cent solution of *sodium bicarbonate*; soluble in *dilute hydrochloric acid*.

Standards : Sulphafurazole is N^1-(3,4-dimethyl-isoxazol-5-yl) sulphanilamide. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{11}H_{13}N_3O_5S$, calculated with reference to the dried substance.

Identification : (A) To a suspension of about 20 mg in 5 ml of *water* add dropwise *sodium hydroxide solution* until solution is complete, then add a few drops of *copper sulphate solution*; a green colour develops and then a greenish-blue precipitate is produced.

(B) Complies with **Identification** test (B) described under Succinylsulphathiazole.

Melting range : Between 194° and 198°, Appendix 5.11.

Clarity and colour of solution : Dissolve 1.0 g in sufficient *dilute hydrochloric acid* to produce 20.0 ml. The resulting solution is clear and the colour is not more intense than that of a mixture of 0.6 ml of *ferric chloride C.S.*, 0.15 ml of *copper sulphate C.S.* and sufficient *water* to produce 20.0 ml.

Acidity : Heat 1.0 g for five minutes at 70° with 50 ml of *carbon dioxide-free water*, cool quickly at 20°, filter and

titrate 25 ml of the filtrate to pH 7.0 with *0.1N sodium hydroxide*, not more than 0.25 ml of *0.1N sodium hydroxide* is required.

Arsenic : Not more than 10 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million determined on 1.0 g by Method C on a solution prepared by dissolving 0.5 g in 5 ml of *N sodium hydroxide* and 20 ml of water, Appendix 3.2.4.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel H* as the coating substance and a mixture of 15 volumes of *n-butyl alcohol* and 3 volumes of *N ammonia* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in a mixture of 9 volumes of *alcohol* and 1 volume of *strong ammonia solution* containing (1) a 0.1 per cent w/v of the substance being examined and (2) 0.0005 per cent w/v of *sulphanilamide*. After removal of the plate, heat it at 105° for ten minutes and spray with a 0.1 per cent w/v *4-dimethylaminobenzaldehyde* in *alcohol* containing 1 per cent v/v of *hydrochloric acid*. Any spot in the chromatogram obtained with solution (1) other than the principal spot, is not more intense than the spot in the chromatogram obtained with solution (2).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g, dissolve in 50 ml of *acetone* and titrate with *0.1N tetrabutylammonium hydroxide* using a 0.3 per cent w/v solution of *thymol blue* in *methyl alcohol* as indicator and taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination and make any necessary correction. Each ml of *1.0N tetrabutylammonium hydroxide* is equivalent to 0.02673 g of $C_{11}H_{13}N_3O_3S$.

Storage : Store in well-closed, light-resistant containers.

Sulphafurazole Tablets

Category : Antibacterial.

Dose : Sulphafurazole. In the treatment of systemic infection; initial dose, 3 g, subsequent doses, upto 6 g daily, in divided doses. In the treatment of infections of urinary tract: initial dose 2 g; subsequent doses, upto 4 g daily, in divided doses.

Usual strength : 0.5 g.

Standards : Sulphafurazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sulphafurazole, $C_{11}H_{13}N_3O_3S$.

Identification : (A) Shake a quantity of the powdered tablets equivalent to 0.2 g of Sulphafurazole with 10 ml of *acetone*, filter, and evaporate the filtrate to dryness; the residue, after drying for two hours at 105°, melts at about 196°, Appendix 5.11 and complies with **Identification** test (A) described under Sulphafurazole.

(B) Extract a quantity of the powdered tablets equivalent to 50 mg of Sulphafurazole with 2 ml of warm *dilute hydrochloric acid* and filter. The filtrate complies with **Identification** test (B) described under Succinylsulphathiazole.

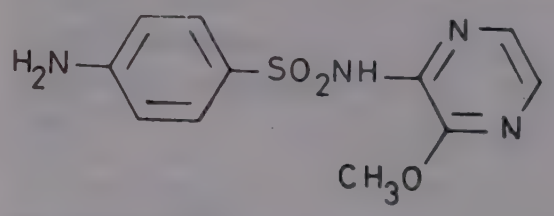
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Carry out the **Assay** described under Sulphafurazole, using an accurately weighed quantity of the powder equivalent to 0.5 g of Sulphafurazole dissolved as completely as possible in 50 ml of *acetone*.

Storage : Store in well-closed, light-resistant containers.

Sulphalene

Sulphamethopyrazine; Sulphamethoxypyrazine



$C_{11}H_{12}N_4O_3S$ Mol. Wt. 280.29

Category : Antibacterial.

Dose : Initial, 800 mg by mouth; subsequent doses, 200 mg daily.

Description : White or yellowish-white, crystalline powder; almost odourless; taste, slightly bitter.

Standards : Sulphalene is N^1-(2-methoxy-3-pyrazinyl) sulphanilamide. It contains not less than 99.0 per cent of $C_{11}H_{12}N_4O_3S$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *sulphalene R.S.*, Appendix 5.15 B.

(B) It gives the reaction of *primary aromatic amines*, Appendix 3.1, giving an orange-red precipitate.

Melting range : Between 180° and 183°, Appendix 5.11.

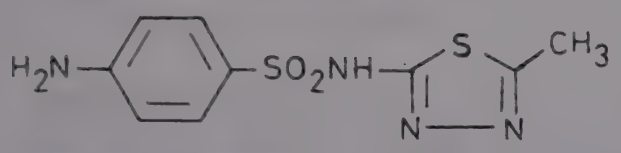
Acidity : Complies with the requirements stated under Sulphadoxine.

Heavy metals; Related substances; Sulphated ash and Loss on drying : Complies with the requirements stated under Sulphadimethoxine.

Assay : Carry out the **Assay** described under Sulphadimethoxine. Each ml of *0.1M sodium nitrite* is equivalent to 0.02803 g of $C_{11}H_{12}N_4O_3S$.

Storage : Store in well-closed, light-resistant containers.

Sulphamethizole



$C_9H_{10}N_4O_2S_2$ Mol. Wt. 270.31

Category : Antibacterial.

Dose : 100 to 200 mg every four to six hours.

Description : Colourless crystals or white or creamy white, crystalline powder; odourless or almost odourless; taste, slightly bitter.

Solubility : Very slightly soluble in *water*, in *chloroform* and in *solvent ether*; freely soluble in solutions of the alkali hydroxides, and in dilute mineral acids; sparingly soluble in *alcohol*.

Standards : Sulphamethizole in N^1-(5-methyl-1,3,-4-thiadiazol-2-yl) sulphanilamide. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_9H_{10}N_4O_2S_2$, calculated with reference to the dried substance.

Identification : (A) The *Infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to those in the spectrum of *sulphamethizole R.S.*, Appendix 5.15 B.

(B) It gives the reactions of *primary aromatic amines*, Appendix 3.1, giving an orange-red precipitate which darkens on standing.

Melting range : Between 208° and 211°, Appendix 5.11.

Clarity and colour of solution : Dissolve 1.0 g in sufficient *2N hydrochloric acid* to produce 20.0 ml. The resulting solution is clear and not more intensely coloured than a mixture of 2.0 ml of *ferric chloride C.S.*, 0.5 ml of *cobalt chloride R.S.* and sufficient *hydrochloric acid (1 per cent w/v)* to produce 20.0 ml.

Acidity : Heat 1.0 g with 50 ml of *carbon dioxide-free water* at about 70° for five minutes, cool quickly to 20°, and filter; titrate 25 ml of the filtrate with *0.1N sodium hydroxide* to pH 7.0; not more than 0.5 ml of *0.1N sodium hydroxide* is required.

Arsenic : Not more than 10 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method C on a solution prepared by dissolving 0.5 g in 5 ml of *N sodium hydroxide* and 20 ml of *water*, Appendix 3.2.4.

Related substances : Complies with the test described under Sulphafurazole.

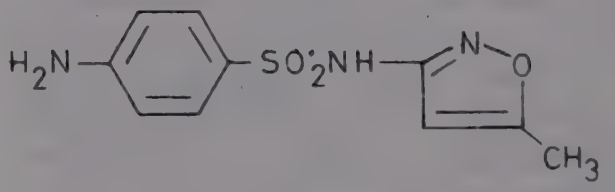
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Carry out the **Assay** described under Succinylsulphathiazole. Each ml of *0.1M sodium nitrite* is equivalent to 0.02703 g of $C_9H_{10}N_4O_2S_2$.

Storage : Store in well-closed, light-resistant containers.

Sulphamethoxazole



$C_{10}H_{11}N_3O_3S$ Mol. Wt. 253.28

Category : Antibacterial.

Dose : Initial, 2 g, then 1 g two or three times daily.

Description : White or yellowish-white, crystalline powder; almost odourless; taste, slightly bitter.

Solubility : Very slightly soluble in *water*, slightly soluble in *alcohol*; soluble in *acetone* and in solutions of alkali hydroxides.

Standards : Sulphamethoxazole is N^1-(5-methyl-isoxazol-3-yl) sulphanilamide. It contains not less than 99.0 per cent of $C_{10}H_{11}N_3O_3S$, calculated with reference to the dried substance.

Identification : (A) It gives the reactions of *primary aromatic amines*, Appendix 3.1.

(B) Dissolve 5 mg in 0.5 ml of *2N sodium hydroxide* and add 5 ml of *water*. Add 0.1 g of *phenol*, heat to boiling, cool, and add 1 ml of *chlorinated soda solution*; a golden-yellow colour is immediately produced and persists.

Melting range : Between 169° and 172°, Appendix 5.11.

pH : Between 4.0 and 6.0, determined in a 10 per cent w/v suspension in *water*, Appendix 5.10.

Heavy metals : Not more than 10 parts per million, determined by Method A, Appendix 3.2.4, on a solution prepared by dissolving 2 g in 5 ml of *sodium hydroxide solution* and 20 ml of *water*.

Related substances : Complies with the test described under Sulphadimethoxine.

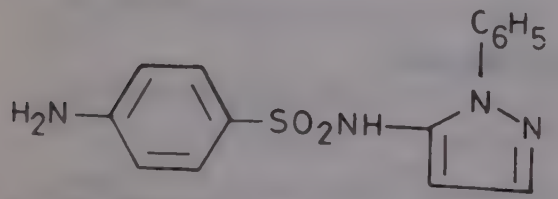
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02533 g of $C_{10}H_{11}N_3O_3S$.

Storage : Store in well-closed, light-resistant containers.

Sulphaphenazole



$C_{15}H_{14}N_4O_2S$ Mol. Wt. 314.36

Category : Antibacterial.

Dose : Initial dose, 1 g every twelve hours, for two days; subsequent dose, 500 mg every twelve hours for three to five days.

Description : White or almost white crystalline powder; odourless.

Solubility : Very slightly soluble in *water*, in *alcohol*, in *solvent ether* and in *chloroform*· soluble in solutions of alkali hydroxides and in mineral acids.

Standards : Sulphaphenazole is N^1-(1-phenylpyrazole-5-yl) sulphanilamide. It contains not less than 99.0 per cent of $C_{15}H_{14}N_4O_2S$, calculated with reference to the dried substance.

Identification : (A) Heat 10 mg in a dry tube; a brown colour is produced; on further heating, yellow fumes are evolved and the odour of sulphur dioxide is produced.

(B) Boil 0.2 g under a reflux condenser for 45 minutes with 25 ml of *sulphuric acid*, cool in ice, add 50 ml of *water*, make alkaline to litmus paper with *sodium hydroxide solution*. Extract with two successive quantities, each of 30 ml of *solvent ether*; to the combined extracts add 20 ml of a saturated solution of *picric acid* in *solvent ether*, a precipitate is formed which, after washing with *solvent ether* and drying, melts at about 152°, Appendix 5.11.

(C) Dissolve, 50 mg in 2 ml of warm *dilute hydrochloric acid*, cool in ice, add 2 ml of *sodium nitrite solution*; pour the solution in 2 ml of *β-naphthol solution* containing 1 g of *sodium acetate*; an orange precipitate is produced.

Melting range : Between 178° and 183°, Appendix 5.11.

Acidity : Heat 2.5 g with 90 ml of *water* for five minutes at 70°, cool, filter, and dilute the filtrate to 100 ml with *water*; 20 ml of this solution requires for neutralization, not more than 0.1 ml of *0.1N sodium hydroxide solution* using *phenolphthalein solution* as indicator.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined by Method A, Appendix 3.2.4, on a solution prepared by dissolving 1.0 g in 5 ml of *N sodium hydroxide* and 20 ml of *water*, Appendix 3.2.4.

Iron : Ignite 2.0 g with 1 g *anhydrous sodium carbonate*, cool, dissolve the residue in 5 ml of *hydrochloric acid*, and dilute to 35 ml with *water*. The resulting solution complies with the *limit test for iron*, Appendix 3.2.5.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03144 g of $C_{15}H_{14}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphaphenazole Tablets

Category : Antibacterial.

Dose : Sulphaphenazole, initial dose, 1 g every twelve hours for two days; subsequent dose, 0.5 g every twelve hours for three days.

Usual strength : 500 mg.

Standards : Sulphaphenazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Sulphaphenazole, $C_{15}H_{14}N_4O_2S$.

Identification : Mix a quantity of the powdered tablets, equivalent to about 1.5 g of Sulphaphenazole, with 30 ml

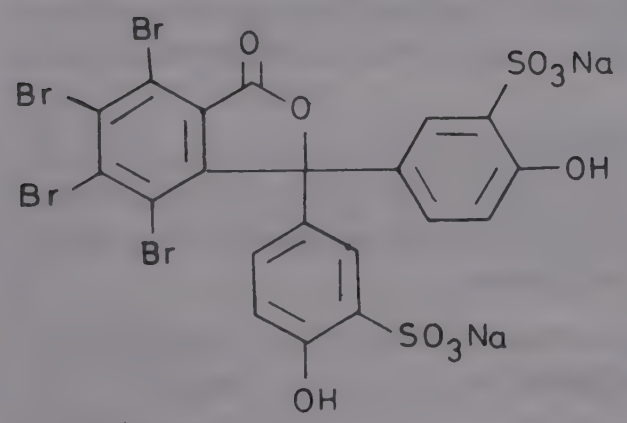
acetone, and heat under a reflux condenser for 5 minutes; filter, evaporate the filtrate on a water-bath to a volume of about 10 ml, add 40 ml of *water*, heat for a further 15 minutes, and cool in ice; a precipitate is formed which, after washing with *water* and drying at 105°, complies with the **Identification** tests described under Sulphaphenazole.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Sulphaphenazole and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.03144 g of $C_{15}H_{14}N_4O_2S$.

Storage : Store in well-closed, light-resistant containers.

Sulphobromophthalein Sodium



$C_{20}H_8Br_4Na_2O_{10}S_2$ Mol. Wt. 837.99

Category : Diagnostic acid (hepato-biliary function determination).

Dose : By intravenous injection, 2 to 5 mg per kg of body weight.

Description : White, crystalline powder; odourless; taste, bitter; hygroscopic.

Solubility : Soluble in *water*; practically insoluble in *alcohol*, and in *acetone*.

Standards : Sulphobromophthalein Sodium is disodium 5,5′-(4,5,6,7-tetrabromo-1,3-dihydro-3-oxo-isobenzofuran-1,1-diyl)bis(2-hydroxybenzenesulphonate). It contains not less than 7.4 per cent and not more than 8.2 per cent of sulphur, S, and not less than 36.0 per cent and not more than 39.0 per cent of bromine, Br, both calculated with reference to the dried substance.

Identification : (A) Mix 0.1 g with 0.5 g of *sodium carbonate* and ignite until thoroughly charred. Cool, add 5 ml of hot *water* and heat for five minutes on a water-bath and filter; the solution gives the reactions of *bromides*, Appendix 3.1.

(B) *Extinction* of a 1-cm layer of a 0.0005 per cent w/v solution in *0.05N sodium hydroxide* at 580 nm, about 0.4, Appendix 5.15 A.

(C) A solution (1 in 20) gives the reactions of *sodium*. Appendix 3.1.

Clarity and colour of solution : 0.2 g dissolves completely in 10 ml of *water* yielding a clear and colourless solution.

Halide ions : To 5 ml of a 1.0 per cent w/v solution add 1 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; no more than a slight opalescence is produced.

Sulphate : To 10 ml of a 0.2 per cent w/v solution add five drops of *dilute hydrochloric acid*, boil, and add 1 ml of *barium chloride solution*; the hot solution remains clear for two minutes.

Calcium : Add 5.0 g in a platinum dish until free from carbon, cool, add 1 ml of *hydrochloric acid* add 10 ml of *water*, heat on a water-bath for five minutes, add 1 g of *ammonium sulphate* and 4 ml of *dilute ammonia solution*, and heat for a further five minutes. Transfer the contents of the dish to a flask with the aid of 50 ml of *water*, add 20 ml of *strong ammonia solution*, dilute to 100 ml with *water*, add 0.3 ml of *sodium sulphide solution*, 1 ml of *potassium cyanide solution*, and 74 ml of *ethyl alcohol*, and titrate with *0.01M disodium ethylenediamine tetraacetate*, using 0.1 g of a mixture of one part of *methyl thymol blue* and 99 parts of *potassium nitrate* as indicator and titrating to a faint grey colour. Not more than 6.25 ml of *0.01M disodium ethylenediamine tetraacetate* is required.

Loss on drying : Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : (1) *For sulphur*—Burn 0.2 g by the *oxygen-flask method*, Appendix 3.3.6, using a 1000-ml glass-stoppered flask and a mixture of 30 ml of *water* and 0.5 ml of *strong hydrogen peroxide solution* as the absorbing liquid. When the process is complete, add 2 ml of *hydrochloric acid*, dilute to 250 ml with *water*, heat to boiling, and slowly add 10 ml of *barium chloride solution*. Heat on a water-bath for one hour, filter, wash the precipitate with *water*, dry, and ignite at a temperature of about 600° until, after further ignition, two successive weighings do not differ by more than 0.2 per cent of the weight of the residue. Each g of residue is equivalent to 0.1374 g of Sulphur.

(2) *For bromine*—Burn 0.2 g by the *oxygen-flask method*, Appendix 3.3.6, using a 1000-ml glass-stoppered flask and a mixture of 10 ml of *0.1N sodium hydroxide*, 0.5 ml of *strong hydrogen peroxide solution* and 10 ml of *water* as the absorbing liquid. When the process is complete, boil the solution for five minutes, cool, acidify with *dilute nitric acid*, add 20 ml of *0.1N silver nitrate*, shake, and titrate the excess of silver nitrate with *0.1N*

ammonium thiocyanate, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.007991 g of Bromine.

Storage : Store in tightly-closed containers.

Sulphobromophthalein Sodium Injection

Category : Diagnostic aid (hepato-biliary function determination).

Dose : Sulphobromophthalein Sodium, 2 to 5 mg per kg of body weight.

Usual strength : 50 mg per ml.

Description : Clear, colourless or almost colourless solution.

Standards : Sulphobromophthalein Sodium Injection is a sterile solution of Sulphobromophthalein Sodium in Water for Injection. It contains not less than 94.0 per cent and not more than 106.0 per cent of the stated amount of $C_{20}H_8Br_4Na_2O_{10}S_2$.

Identification : (A) To a few ml add a few drops of *N sodium hydroxide*; an intense purple colour is produced which disappears on adding acid.

(B) To a volume equivalent to 50 mg of Sulphobromophthalein Sodium add 0.25 g of *sodium carbonate*, evaporate to dryness and ignite; the residue gives the reactions of *bromides*, Appendix 3.1.

pH : Between 5.0 and 6.5, determined by using a suitable agar-potassium nitrate salt bridge, Appendix 5.10.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using a volume equivalent to 2.5 mg of Sulphobromophthalein Sodium per kg of the rabbit's weight diluted to not more than 5 ml with *pyrogen-free saline solution*.

Other requirements : Complies with the requirements stated under Injections.

Assay : Measure accurately a volume equivalent to 0.1 g of Sulphobromophthalein Sodium and dilute to 500.0 ml with *water*. Pipette 5.0 ml into 200-ml graduated flask and dilute to volume with a 1.0 per cent w/v solution of *sodium carbonate*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 580 nm using the sodium carbonate solution as the blank, Appendix 5.15 A. Calculate the content of $C_{20}H_8Br_4Na_2O_{10}S_2$ from the *extinction* obtained by carrying out the Assay simultaneously on 0.1 g, accurately weighed, *sulphobromophthalein sodium R.S.* and from the declared content of $C_{20}H_8Br_4Na_2O_{10}S_2$ in the *sulphobromophthalein sodium R.S.*

Storage : Store in single-dose containers.

Syrups

Syrups are oral liquids which are sweet and viscid and often contain added flavouring and colouring agents and into which medicaments are incorporated. Traditionally, syrups are concentrated aqueous solutions of sucrose or other sugars to which certain polyhydric alcohols such as glycerin or sorbitol may be added to inhibit crystallisation or to modify solubilisation, taste and other vehicle properties. Sugarless syrups may contain sweetening agents such as saccharin and sorbitol and thickening agents such as cellulosic gums.

Syrups may contain a small concentration of alcohol as a preservative or as a solvent to incorporate flavouring agents. Antimicrobial agents may be present to prevent the growth of bacteria, yeasts and moulds.

A near-saturated solution of sucrose in purified water is known as syrup or simple syrup. When the syrup contains a medicament, the product is described as a medicated syrup.

Storage : Syrups should be stored in tightly-closed containers at temperatures not exceeding 30°.

Tablets

Tablets are solid dosage forms, each containing a unit dose of one or more medicaments. They are generally intended for oral administration.

Some tablets are swallowed whole or after being chewed, some are dissolved or dispersed in water before administration, and some are retained in the mouth where the medicament is released.

The vast majority of all tablets manufactured are made by compression of uniform volumes of powders or granules by applying high pressures and utilising steel punches and dies. The particles to be compressed consist of one or more medicaments, with or without added substances such as diluents, binders, disintegrating agents, lubricants, and substances capable of modifying the behaviour of the medicaments in the digestive tract.

Tablets can be manufactured in a variety of sizes, shapes and surface markings. Compressed tablets are flat or convex and may have lines or break-marks, a symbol or any other markings. They are sufficiently hard to withstand handling without crumbling or breaking.

Tablets are uncoated except where the individual monographs permits the use of coating, in which case the statement "The tablets may be coated" provides the option for the tablets to be coated or uncoated, or directs the use of coating, in which case the individual monograph states "The tablets are coated". Unless otherwise directed, tablets may be coated in one of different ways given below:

Added substances : Diluents, disintegrating agents, moistening agents, absorbents, lubricants and other added substances must be innocuous and therapeutically inert in the quantities present.

Uncoated tablets : Uncoated tablets consist of single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of parallel layers obtained by successive compression of particles of different compositions. No treatment is applied to the tablets after compression. Uncoated tablets do not contain added substances specifically intended to modify the release of the medicament in the digestive fluids. The addition of colouring or flavouring agents to uncoated tablets is not official, unless permitted in the individual monograph.

Coated tablets : Coated tablets are tablets covered with one or more layers of mixtures of various substances such as resins, gums, inactive and insoluble fillers, sugars, waxes, etc. The coating may also contain medicaments. In compression-coated tablets the coating is applied by compressing around the tablets, granules prepared from lactose-calcium phosphate or other suitable substances or mixtures of such substances. In film-coated tablets, the coating comprising less than 10 per cent by weight of the finished tablet, consist of a suitable synthetic or natural film-forming material applied by deposition from a solution of the material in a suitable solvent.

Coated tablets may contain one or more colouring agents permitted under the Drugs and Cosmetics Rules, 1945.

Enteric-coated tablets : Enteric-coated tablets are tablets covered with one or more layers of coatings intended to resist the gastric fluid but permit disintegration in the intestinal fluid. For this purpose substances such as cellulose acetate phthalate and anionic copolymers of methacrylic acid and its esters are used in the coating.

Soluble tablets : Soluble tablets are uncoated tablets which dissolve in water to produce a solution which may be slightly opalescent due to the excipients used in the manufacture of the tablets.

Dispersible tablets : Dispersible tablets are uncoated tablets that produce a uniform dispersion in water.

Requirements of tests : Tablets comply with the following requirements:

1. *Appearance*—When a broken section of an uncoated tablet is examined under a lens, either a relatively uniform texture (single-layer tablet) or a stratified structure (multi-layer tablet) is seen; there are no signs of coating.

Coated tablets have a smooth and often coloured, surface. Sugar-coated tablets are usually polished; when a broken section of a coated tablet is examined under a lens, a core surrounded by a continuous layer of a different structure is seen.

2. *Content of active ingredient in tablets*—Determine the amount of active ingredient by the method in the Assay; calculate, if necessary, the amount of active ingredient in the tablets taken for the assay and divide by the number of tablets. The result lies within the range for the content of active ingredient stated in the monograph. These ranges are expressed in terms of the weight stated on or indicated by the prescription or order and have been framed to allow for all permissible variations, including that of the active ingredient itself and that due to the process of manufacturing the tablets. When a biological assay is specified, the standards for the content of active ingredient are set out in the Assay. The ranges are based on the requirement that 20 tablets, or such other number as may be indicated in the monograph, are used in the Assay In circumstances where 20 tablets cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1.

TABLE 1

Weight of medicament in each tablet	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

The requirements of Table 1 apply when the stated limits are between 90 and 110 per cent. For limits less than 90 or greater than 110 per cent, proportionately larger allowances should be made.

3. *Uniformity of weight*—Uncoated tablets comply with the following tests: Weigh twenty tablets selected at

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random and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation shown in Table 2, and none deviates by more than twice that percentage.

TABLE 2

Average Weight of Tablet	Percentage Deviation
80 mg or less	10
More than 80 mg and less than 250 mg	7.5
250 mg or more	5

4. *Uniformity of content*—Comply with the requirements for uniformity of content specified in the individual monograph.

5. *Disintegration*—Uncoated tablets except for chewable tablets, comply with the *disintegration test for tablets*, Appendix 5.6.1. The tablets disintegrate within fifteen minutes unless otherwise indicated in the monograph.

Coated tablets except for chewable tablets comply with the *disintegration test for tablets*, Appendix 5.6.1, the apparatus being operated for sixty minutes, unless otherwise specified. Remove the assembly from the liquid. The tablets pass the test if all six have disintegrated. If any of the tablets have not disintegrated repeat the test on a further six tablets, replacing the *water* in the beaker with *0.1N hydrochloric acid*. The tablets then pass the test if all six tablets have disintegrated in the acid medium.

Enteric-coated tablets comply with the *disintegration test for enteric-coated tablets*, Appendix 5.6.1.

Soluble tablets and Dispersible tablets disintegrated within three minutes when examined by the *disintegration test for tablets*, Appendix 5.6.1, but using *water* at 19° to 21°, unless otherwise stated in the individual monograph.

General Requirements

1. Storage : Tablets should be stored in containers which afford protection against breakage or crushing, access of moisture, contamination and to the extent possible deterioration.

2. Labelling : The label on the container states (1) the name of the tablets; (2) the quantity of the active ingredient or ingredients contained in each tablet; (3) "film-coated" or "enteric-coated" for tablets coated in either manner.

Talc

Purified Talc, Talcum

Category : Pharmaceutical aid (dusting powder).

Description : Very fine, white or almost white powder; free from grittiness, readily adheres to the skin, unctuous to the touch; odourless; tasteless.

Solubility : Practically insoluble in *water* and in dilute solutions of acids or alkalis.

Standards : Talc is a powdered, purified, natural hydrated magnesium silicate containing a small amount of aluminium silicate.

Identification : (A) Mix 0.5 g with about 0.2 g of *anhydrous sodium carbonate* and 2 g of *anhydrous potassium carbonate*, and heat the mixture in a platinum crucible until fusion is complete. Cool and transfer the fused mixture to a dish or beaker with the aid of about 50 ml of hot *water*. Add *hydrochloric acid* to the liquid until it ceases to cause effervescence, then add 10 ml more of the acid, and evaporate the mixture to dryness on a water-bath. Cool, add 20 ml of *water*, boil, and filter the mixture. Dissolve in the filtrate about 2 g of *ammonium chloride*, and add 5 ml of *dilute ammonia solution*. Remove by filtration any precipitate which may form, and add *disodium hydrogen phosphate solution* to the filtrate; a white crystalline precipitate of magnesium ammonium phosphate separates.

(B) In a metal crucible melt 0.5 g with 4 g of *anhydrous sodium carbonate*, dissolve the melt in hot *water* and filter. To the filtrate add sufficient *hydrochloric acid* to produce a strong acid reaction and evaporate to dryness. The residue gives the reactions of *silicates*, Appendix 3.1.

Acidity or Alkalinity : Shake 5.0 g with 25 ml of *carbon dioxide-free water* for one minute, filter and add to the filtrate 0.5 ml of *bromothymol blue solution*; the solution is not acid and requires not more than 0.3 ml of *0.1N hydrochloric acid* to make it acid.

Water-soluble substances : Evaporate 25 ml of the filtrate obtained in the preceding test and dry to constant weight at 105°; the residue does not weigh more than 10 mg.

Acid-soluble substances : Not more than 2.0 per cent, determined by the following method: Digest 2.0 g with 40 ml of *dilute hydrochloric acid* for fifteen minutes. Filter, evaporate the filtrate; to the residue add 0.1 ml of *sulphuric acid* and ignite to constant weight.

Iron : Boil 5 g with 25 ml of *water* for thirty minutes, replacing any water lost by evaporation, and filter. The filtrate, after the addition of 5 ml of *nitric acid* and dilution to 50 ml with *water*, complies with the *limit test for iron*, Appendix 3.2.5.

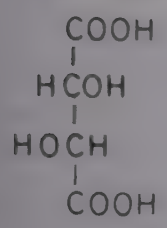
Carbonates : 1 g added to 20 ml of *dilute hydrochloric acid* shows no effervescence.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 180° for one hour, Appendix 5.8.

Organic compounds : The residue obtained in the test for **Loss on drying** is not more than slightly yellow or grey.

Storage : Store in well-closed containers.

Tartaric Acid



$C_4H_6O_6$ Mol. Wt. 150.09

Category : Pharmaceutical aid (buffering agent).

Description : Colourless or translucent crystals or white or almost white, fine granules or crystalline powder; odourless or nearly odourless; taste, strongly acidic.

Solubility : Very soluble in *water*; freely soluble in *alcohol*.

Standards : Tartaric Acid is L-tartaric acid [(2*R*,3*R*)-2,3-dihydroxysuccinic acid]. It contains not less than 99.5 per cent of $C_4H_6O_6$, calculated with reference to the dried substance.

Identification : (A) Ignite a few mg; it gradually decomposes giving off an odour resembling that of burning sugar (distinction from citric acid).

(B) A solution (1 in 20) gives the reactions of *tartrates*, Appendix 3.1.

Clarity and colour of solution : A 10.0 per cent w/v solution is clear; 20.0 ml of the solution is not more intensely coloured than a mixture of 0.6 ml of *ferric chloride C.S.*, 0.15 ml of *cobalt chloride C.S.* and sufficient 1.0 per cent w/v solution of *hydrochloric acid*, to produce 20.0 ml.

Specific optical rotation : Between +12.2° and +13.0°, determined in a 20.0 per cent w/v solution, Appendix 5.12.

Oxalate : Neutralise 10 ml of a 10.0 per cent w/v solution with *dilute ammonia solution*, add 0.1 ml of *dilute acetic acid* and 10 ml of *calcium sulphate solution*; no opalescence is produced within twenty minutes.

Sulphates : To 10 ml of a 1.0 per cent w/v solution add three drops of *hydrochloric acid* and 1 ml of *barium chloride solution*; no turbidity is produced.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 3.2.4.

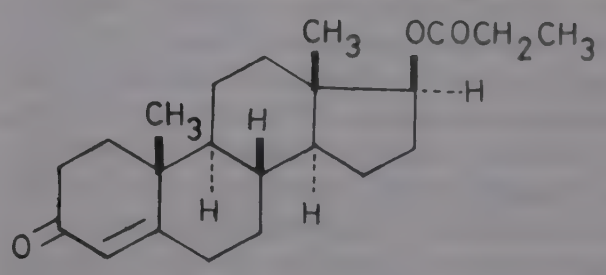
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.15 g, dissolve in 25 ml of *water* and titrate with *0.1N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.007505 g of $C_4H_6O_6$.

Storage : Store in well-closed containers.

Testosterone Propionate



$C_{22}H_{32}O_3$ Mol. Wt. 344.49

Category : Androgen.

Dose : By intramuscular injection, 5 to 25 mg, once or twice weekly.

Description : White or creamy-white, crystalline powder or colourless or slightly yellowish-white crystals; odourless.

Solubility : Practically insoluble in *water*, freely soluble in *acetone*, in *alcohol*, in *dioxan* and in *solvent ether*, soluble in vegetable oils.

Standards : Testosterone Propionate is 3-oxo-4-androsten-17β-yl propionate. It contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of $C_{22}H_{32}O_3$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *testosterone propionate R.S.*, Appendix 5.15 B.

(B) Dissolve 1 mg in 1 ml of *sulphuric acid*. The solution is colourless or yellowish. Add 4 ml of *water* and then 6 ml of *sulphuric acid*. The solution becomes greenish-yellow with a green fluorescence.

(C) Boil 25 mg with 2 ml of a 1.0 per cent w/v solution of *potassium hydroxide* in *methyl alcohol* under a reflux

condenser for one hour. Cool, add 10 ml of *water*, filter, and wash the precipitate with *water* until the washings are neutral to litmus. The precipitate, after drying "in vacuo at 60°" for three hours, melts at about 152°, Appendix 5.11.

Melting range : Between 118° and 123°, Appendix 5.11.

Specific optical rotation : Between +83° and +90°, determined in a 1.0 per cent w/v solution in *dioxan*, Appendix 5.12.

Clarity and colour of solution : A 5.0 per cent w/v solution in *ethyl alcohol* is clear and not more intensely coloured than a mixture of 1.2 ml of *ferric chloride C.S.*, 0.5 ml of *cobalt chloride C.S.*, 0.2 ml of *copper sulphate C.S.*, and sufficient *hydrochloric acid (1 per cent w/v)* to produce 100.0 ml.

Free testosterone : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3., using *silica gel G* as the coating substance and a mixture of 92 volumes of *ethylene chloride*, 8 volumes of *methyl alcohol* and 0.5 volumes of *water* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in a mixture of 9 volumes of *chloroform* and 1 volume of *methyl alcohol* containing (1) 2.0 per cent w/v of the substance being examined and (2) 0.020 per cent w/v of *testosterone R.S.* After removal of the plate allow it to dry in air until the odour of the solvent is no longer detectable, heat at 110° for ten minutes and spray the hot plate with a 10 per cent v/v solution of *sulphuric acid* in *alcohol*. Heat at 110° for a further ten minutes and examine under an ultra-violet lamp having a maximum output at about 366 nm. The spot in the chromatogram obtained with solution (2) is more intense than any corresponding spot in the chromatogram obtained with solution (1).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for two hours, Appendix 5.8.

Assay : Weigh accurately about 10 mg and dissolve in sufficient *ethyl alcohol* to produce 100.0 ml. Dilute 5.0 ml to 50.0 ml with *ethyl alcohol*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 241 nm, using *ethyl alcohol* in the blank, Appendix 5.15 A. Calculate the content of $C_{22}H_{32}O_3$, taking 485 as the E(1 per cent, 1-cm) at the maximum at about 241 nm.

Storage : Store in well-closed, light-resistant containers.

Testosterone Propionate Injection

Category : Androgen.

Dose : Testosterone Propionate, by intramuscular injection, 5 to 25 mg once or twice weekly.

Usual strength : 10 mg per ml.

Standards : Testosterone Propionate Injection is a sterile solution of Testosterone Propionate in Ethyl Oleate or other suitable ester, in a suitable fixed oil, or in any mixture of these. It contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{22}H_{32}O_3$.

Identification : Dissolve a volume equivalent to 50 mg of Testosterone Propionate in 8 ml of *light petroleum (boiling range 40° to 60°)* and extract with three quantities, each of 8 ml, of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (boiling range 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand for two hours in ice, and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests :

(A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *testosterone propionate R.S.*, Appendix 5.15 B.

(B) Complies with the test for *identification of steroids*, Appendix 3.3.11, using *solvent III* and *mobile phase F*.

Other requirements : Complies with the requirements stated under Injections.

Assay : Carry out the **Assay** described under Nandrolone Decanoate Injection, using an accurately measured volume equivalent to 0.1 g of Testosterone Propionate. Calculate the content of $C_{22}H_{32}O_3$ from the *extinction* obtained by repeating the operation using a 0.006 per cent w/v solution of *testosterone propionate R.S.* in *chloroform* and beginning at the words "to 5.0 ml add 10 ml....".

Storage : Store in single dose or multiple dose, light-resistant containers.

Tetanus Antitoxin

Category : Immunising agent.

Dose : By subcutaneous or intramuscular injection, prophylactic, not less than 1500 International Units; therapeutic, not less than 50,000 International Units.

Description : Almost colourless or very faintly yellow liquid.

Standards : Tetanus Antitoxin is a preparation containing the specific antitoxic globulins or their derivatives obtained by purification from native serum and having the specific power of neutralising the toxin formed by *Clostridium tetani*. It has a potency of not less than 1000 International Units per ml when intended for prophylactic use and not less than 3000 International Units per ml when intended for therapeutic use.

Identification : It specifically neutralises and renders the toxins formed by *Clostridium tetani* harmless to susceptible animals.

Other requirements : Complies with the requirements for general tests, stated under Antisera.

Potency : Carry out the *determination of potency of tetanus antitoxin*, Appendix 2.19.

Storage : Store in containers, protected from light, at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling : The label on the container states (1) the number of Units per ml; (2) whether the preparation is for prophylactic use or for therapeutic use; (3) the recommended dose; (4) the storage conditions; (5) the date after which it is not intended to be used.

Tetanus Vaccine (Adsorbed)

Tetanus Toxoid (Adsorbed)

Category : Active immunising agent.

Dose : By deep intramuscular injection, two injections of 0.5 ml at an interval of four to six weeks, followed by a third, reinforcing dose of 0.5 ml, six to eight months later.

Standards : Tetanus Vaccine (Adsorbed) is a sterile suspension of purified tetanus vaccine, adsorbed on a mineral carrier such as aluminium hydroxide or aluminium phosphate. The vaccine is prepared from the toxin produced by the growth in suitable media of the tetanus bacillus *(Clostridium tetani)* by methods which avoid reversibility of the toxoid. The toxin is converted to tetanus formol toxoid by treatment with formaldehyde solution. The toxoid which has a purity of not less than 1000 Lf per mg of protein nitrogen is mixed with a suspension of aluminium hydroxide or aluminium phosphate in a 0.9 per cent w/v solution of Sodium Chloride or other appropriate solution isotonic with blood. The final vaccine contains a suitable preservative other than any of the phenol or cresol groups.

It contains not less than 5 Lf of tetanus toxoid per dose of 0.5 ml.

Description : White or greyish-white, turbid liquid; the mineral carrier may tend to settle down on keeping.

Identification : When injected into suitable laboratory animals, it induces tetanus antitoxin in the circulating blood of the animals.

pH : Between 6.2 and 6.8, Appendix 5.10.

Aluminium, Free formaldehyde, Undue toxicity : Complies with the tests described under Diphtheria and Tetanus Vaccine (Adsorbed).

Thiomersal (if present) : Between 0.005 per cent and 0.02 per cent w/v, Appendix 4.7.

Specific toxicity : Use not fewer than five normal, healthy guinea-pigs weighing between 250 and 350 g, which have been maintained for at least one week on a uniform, unrestricted diet and have not lost weight during this period and which have not previously been treated with any material that will interfere with the test. Weigh the animals separately and record the weights. Inject subcutaneously five times the dose stated on the label, into each of the animals. Weigh all the animals at weekly intervals and on the twenty-first day. None of the animals shall die or show signs of tetanus toxaemia with in 21 days or lose weight at the end of the test. If more than one animal dies from non-specific causes, repeat the test. If an animal dies in the second test, the vaccine fails the test.

Potency : Determine by either of the following two methods : (1) Inject subcutaneously on each of two occasions separated by an interval of not more than four weeks, one-tenth of the stated human dose diluted to 1 ml with *saline solution* into each of ten normal, healthy guinea-pigs weighing between 250 and 350 g. Not later than two weeks after the second injection, collect the serum from each animal and carry out the *biological test for tetanus antitoxin*, Appendix 2.27.

The serum of each of not less than two-thirds of the guinea-pigs contains not less than 0.05 Unit of tetanus antitoxin per ml, or, alternatively, the serum of each of not less than one-third of the guinea-pigs contains not less than 0.5 Unit per ml.

(2) Carry out the *biological assay of adsorbed tetanus vaccine*, Appendix 2.28.

Storage : Store in single-dose or multiple-dose containers at the temperature between 2° and 8° The vaccine should not be frozen.

Labelling : The label on the container states (1) the human dose; (2) the name of the mineral carrier;

(3) the name and proportion of any added preservative; (4) the words "Not to be frozen"; (5) the date after which it is not intended to be used; (6) the storage conditions.

Tetrachloroethylene

$CCl_2:CCl_2$ Mol. Wt. 165.83

Category : Anthelmintic.

Dose : 1 to 3 ml as a single dose.

Description : Clear, colourless, mobile liquid; odour, characteristic and ethereal. It slowly decomposed by light and by contact with various metals in the presence of moisture.

Solubility : Practically insoluble in *water*; miscible with an equal volume of *alcohol*, with *solvent ether*, with *chloroform*, and with *benzene*; dissolves in most of the fixed and volatile oils.

Standards : Tetrachloroethylene contains not less than 99.0 per cent and not more than 99.5 per cent of C_2Cl_4, the remainder consisting of alcohol.

Identification : (A) Boil a few drops with 5 ml of *2N sodium hydroxide* for one minute, cool and add 10 ml of *dilute nitric acid* and five drops of *silver nitrate solution*; a white precipitate is formed which dissolves in *dilute ammonia solution*.

(B) Shake 2 ml with 2 ml of *bromine solution*; the brownish colour disappears within a few minutes when the solution is exposed to sunlight, slowly in diffused light (distinction from chloroform and from carbon tetrachloride).

Distillation range : Not less than 90 per cent distils between 118° and 122°, Appendix 5.3.

Specific gravity : Between 1.603 and 1.615, Appendix 5.19, indicating between 99.0 per cent and 99.5 per cent of C_2Cl_4.

Acidity : In each of two glass-stoppered, 50-ml cylinders, each having an internal diameter of 20 mm, place 10 ml of *water*, two drops of *phenolphthalein solution* and enough *0.01N sodium hydroxide* to produce, after shaking, pink colour of equal intensity. Into one of the cylinders pipette 20 ml of the substance to be examined and shake vigorously. Add *0.01N sodium hydroxide*, dropwise, shaking well after each addition, until the pink colour is reproduced in an intensity equal to that in the cylinder without the Tetrachloroethylene; not more than 0.5 ml of *0.01N sodium hydroxide* is required to produce a pink colour that persists for five minutes.

Chlorides : Shake 25 ml with an equal volume of *water* for five minutes, and allow the liquids to separate completely. To 10 ml of the water layer add five drops of *silver nitrate solution* and one drop of *nitric acid*; any turbidity produced is not more than that produced in 10 ml of *water* to which 0.1 ml of *0.02N hydrochloric acid* has been added.

Free chlorine : Shake 5 ml with a solution of 0.1 g *potassium iodide* in 5 ml of *water* and a few drops of *starch solution* for one minute and allow the liquids to separate completely; both layers are colourless or nearly colourless.

Phosgene : Place 20 ml in a glass-stoppered container, and add 0.1 g of *benzidine*. Stopper the container and allow to stand in the dark for twenty-four hours. The solution shows no turbidity or flocculence.

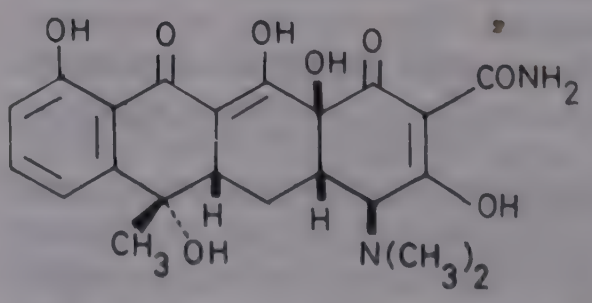
Acetylenic compounds : Place 5 ml in a stoppered cylinder, add 1 ml of *ammoniacal copper nitrate solution* and 5 ml of *ethyl alcohol*, mix, add 0.1 g of *hydroxylamine hydrochloride*, agitate gently and set aside in the dark for fifteen minutes; no orange-red colour is produced.

Readily carbonisable substances : Place 20 ml in a glass-stoppered cylinder that has previously been rinsed with *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4). Add 5 ml of *sulphuric acid* (containing 94.5 to 95.5 per cent w/w of H_2SO_4), shake vigorously for five minutes and allow the two liquids to separate completely; the acid layer is not more coloured than a mixture of 0.1 ml of *cobalt chloride C.S.*, 0.4 ml of *ferric chloride C.S.*, 0.1 ml of *copper sulphate C.S.*, and 4.4 ml of *water*.

Non-volatile matter : Not more than 0.004 per cent w/v, determined by evaporating 50.0 ml and drying the residue at 105° for two hours.

Storage : Store in tightly-closed, light-resistant containers.

Tetracycline



$C_{22}H_{24}N_2O_8$ Mol. Wt. 444.45

Category : Antibacterial.

Dose : 1 to 3 g, daily, in divided doses.

Description : Yellow crystalline powder; odourless.

Solubility : Very slightly soluble in *water*; sparingly soluble in *alcohol*; practically insoluble in *chloroform* and in *solvent ether*; freely soluble in *dilute hydrochloric acid* and in solutions of alkali hydroxides.

Standards : Tetracycline is (4*S*,4a*R*,5a*R*,6*S*,12a*S*)-4-dimethylamino-1,4,4a, 5, 5a,6,11,12a-octohydro-3,6,10, 12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide. It has a potency not less than 975 µg of tetracycline hydrochloride per mg, calculated with reference to the anhydrous substance.

Identification : (A) Complies with **Identification** test (A) described under Oxytetracycline Hydrochloride, using a freshly-prepared 0.05 per cent w/v solution of *tetracycline R.S.* as solution (2).

(B) To about 0.5 mg add 2 ml of *sulphuric acid*; an intense violet colour is produced. Add 1 ml of *water*, the colour changes to deep yellow.

(C) To about 0.5 mg add 2 ml of *sulphuric acid*, mix and add one drop of *ferric chloride test-solution*; a brown to reddish-brown colour is produced.

Specific optical rotation : Between −260° and −280°, determined at 20° in 1.0 per cent w/v solution in *0.1N hydrochloric acid*, Appendix 5.12.

Light absorption : To 10 ml of a 0.01 per cent w/v solution in *0.01N hydrochloric acid*, add 75 ml of *water* and 5 ml of *sodium hydroxide solution*, dilute to 100.0 ml with *water*, mix immediately, and measure the *extinction* of a 1-cm layer at 380 nm exactly 6 minutes after the addition of *sodium hydroxide solution; extinction* at 380 nm, 0.40 to 0.42, calculated with reference to the anhydrous substance, Appendix 5.15 A.

pH : Between 3.5 and 6.0, determined in a 1.0 per cent w/v suspension, Appendix 5.10.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using a coating substance prepared in the following manner: Mix 40 g of *kieselguhr H* with 100 ml of a 5 per cent w/v solution of *disodium ethylenediaminetetraacetate*, previously adjusted to pH 7.5 with *sodium hydroxide solution*. After spreading the plate allow it to dry at room temperature for one hour. Carry out the test in subdued light. Use as the mobile phase a mixture of 1 volume of *water*, 3 volumes of *ethyl acetate*, and 23 volumes of *acetone*. Apply separately to the plate 1 µl of each of six solutions in *methyl alcohol* containing (1) 0.5 per cent w/v of the substance being examined, (2) 0.0025 per cent w/v of *4-epianhydrotetracycline hydrochloride R.S.*, (3) 0.020 per cent w/v of *4-epitetracycline hydrochloride R.S.*, (4) 0.0025 per cent w/v of *anhydrotetracycline hydrochloride R.S.*, (5) 0.005 per cent w/v of *tetracycline hydrochloride R.S. solution*, (6) contains each of the reference substances in the same concentrations as is specified for solutions (2) to (5).

After removal of the plate, dry it in a current of warm air and examine under an ultra-violet lamp having a maximum output at about 365 nm. No spot in the chromatogram obtained with solution (1), other than the principal spot, is more intense than the corresponding spot in the chromatogram obtained with solutions (2) to (5). The test is not valid unless the chromatogram obtained with solution (6) shows four clearly-separated spots.

Undue toxicity : Complies with the test described under Bacitracin, the dose being 0.5 ml of a solution prepared by dissolving a quantity equivalent to 40 mg of anhydrous tetracycline in 2 ml of *0.1N hydrochloric acid* and diluting to 20 ml with *water for injection*.

Water : Not more than 13 per cent w/w, Appendix 3.3.25.

Assay : Weigh accurately a quantity equivalent to about 50 mg of anhydrous tetracycline, dissolve in sufficient *0.1N hydrochloric acid* to produce 50.0 ml. Carry out the *microbiological assay of antibiotics, Method A or B*, Appendix 4.1, and express the result in µg of tetracycline per mg.

Storage : Store in tightly-closed, light-resistant containers.

Labelling : The label on the container states (1) the date after which the contents are not intended to be used; (2) the storage conditions.

Tetracycline Eye Ointment

Tetracycline Hydrochloride Eye Ointment

Category : Antibacterial.

Usual strengths : 5 mg per g; 10 mg per g.

Standards : Tetracycline Eye Ointment contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of Tetracycline Hydrochloride, $C_{22}H_{24}N_2O_8$, HCl.

Identification : Heat a quantity equivalent to about 20 mg of Tetracycline Hydrochloride with 20 ml *methyl alcohol* for twenty minutes, cool in ice, filter and carefully evaporate on a water-bath to dryness; the residue complies with **Identification** test (A) described under Tetracycline Hydrochloride.

Water : Not more than 0.5 per cent w/w, Appendix 3.3.25.

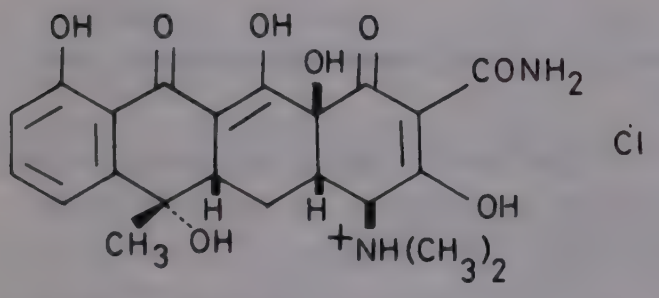
Other requirements : Complies with the requirements stated under Eye Ointments.

Assay : Weigh accurately about 1 g and transfer to a separator. Add 25 ml of *solvent ether*, shake well and extract with five successive quantities, each of 20 ml, of *hydrochloric acid*. Combine the extracts and make upto 200.0 ml with *0.1N hydrochloric acid*. Dilute a suitable volume with *buffer solution, pH 4.5* to produce a solution containing 1 µg per ml of tetracycline hydrochloride. Carry out the *microbiological assay of antibiotics, Method A or B*, Appendix 4.1. Express the result in mg of Tetracycline Hydrochloride, calculated as $C_{22}H_{24}N_2O_8,HCl$ per g.

Storage : Store in well-closed containers, in a cool place.

Labelling : The label on the container states (1) the date after which the contents are not intended to be used; (2) the storage conditions.

Tetracycline Hydrochloride



$C_{22}H_{24}N_2O_8,HCl$ Mol. Wt. 480.90

Category : Antibacterial; anti-amoebic.

Dose : For adults, 1 to 3 g daily, in divided doses; for children 10 to 30 mg per kg of body weight daily, in divided doses.

By intravenous infusion, in a concentration not exceeding 0.1 per cent w/v, 1 to 2 g daily.

Description : Yellow, crystalline powder; odourless; taste, bitter.

Solubility : Freely soluble in *water*, soluble in aqueous solutions of alkali hydroxides and carbonates, slightly soluble in *alcohol*; practically insoluble in *acetone*, in *chloroform* and in *solvent ether*.

Standards : Tetracycline Hydrochloride is (4*S*,-4a*S*, 5a*S*, 6*S*, 12a*S*)-N-(2-carbamoyl-1, 4, 4a, 5,5a,6,-11,12a-octahydro-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxonaphthacen-4-yl) dimethylammonium chloride. It contains not less than 900 µg of $C_{22}H_{24}N_2O_8,HCl$ per mg, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** test (A) described under Oxytetracycline Hydrochloride, using a freshly prepared 0.05 per cent w/v solution of *tetracycline hydrochloride R.S.* in *methyl alcohol* as solution (2).

(B) To 0.5 mg add 2 ml of *sulphuric acid*; purplish-red colour is produced. Add 1 ml of *water*; the colour changes to deep yellow.

(C) A solution (1 in 20) gives the reactions of *chlorides*, Appendix 3.1.

Specific optical rotation : Between −239° and −258°, determined in a 1.0 per cent w/v solution in *0.01N hydrochloric acid*, Appendix 5.12.

Light absorbing impurities : *Extinction* of a 1-cm layer of a 0.2 per cent w/v solution in *0.01N hydrochloric acid* at 430 nm, measured within one hour of introducing the substance to the solvent, not greater than 0.50, Appendix 5.15 A.

Light absorption : Dissolve 10 mg in sufficient *0.01N hydrochloric acid* to produce 100 ml. To 10 ml of the resulting solution add 75 ml of *water*, 5 ml of *5N sodium hydroxide*, and sufficient *water* to produce 100 ml and mix immediately. *Extinction* of a 1-cm layer of the resulting solution at 380 nm, measured exactly six minutes after the addition of the alkali solution, 0.36 to 0.39, Appendix 5.15 A.

pH : Between 1.8 and 3.0, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Heavy metals : Not more than 50 parts per million, determined on 0.4 g by Method B, Appendix 3.2.4.

Related substances : Complies with the test described under Tetracycline.

Undue toxicity : Complies with the test described under Oxytetracycline, the dose being 0.5 ml of a solution containing 2 mg of Tetracycline Hydrochloride per ml.

Sulphated ash : Not more than 0.5 per cent, Appendix 3.2.7.

Loss on drying : Not more than 2.0 per cent, determined on 1.0 g by drying "in vacuo at 60°" for three hours, Appendix 5.8.

Assay : Carry out the *microbiological assay of antibiotics, Method A* or *B*, Appendix 4.1, and express the results in µg of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8,HCl$ per mg.

Tetracycline Hydrochloride intended for parenteral administration complies with the following additional requirements:

Histamine-like substances : Complies with the *test for*

histamine-like substances, Appendix 2.35, using a solution containing 5 mg per ml.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36 volume not exceeding 5 ml containing not less than 5 mg per kg of the rabbit's weight, dissolved in *water for injection*.

Sterility : Complies with the *test for sterility*, Appendix 4.6.

Storage : Store in tightly-closed, light-resistant containers. If it is intended for parenteral administration, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling : The label on the container states (1) the date after which the contents are not intended to be used; (2) the storage conditions; (3) whether or not the contents are intended for parenteral administration.

Tetracycline Capsules

Tetracycline Hydrochloride Capsules

Category : Antibiotic; antibacterial; anti-amoebic.

Dose : Tetracycline Hydrochloride. For adults, 1 to 3 g daily, in divided doses; for children, 10 to 30 mg per kg of body weight daily, in divided doses.

Usual strength : 0.25 g.

Standards : Tetracycline Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of Tetracycline Hydrochloride, $C_{22}H_{24}N_2O_8,HCl$.

Identification : (A) The contents of the capsules comply with **Identification** test (A) described under Doxycycline Hydrochloride, solution (1) being freshly prepared by extracting a quantity of the contents of the capsules equivalent to 10 mg of Tetracycline Hydrochloride with 20 ml of *methyl alcohol*, centrifuging, and using the supernatant liquid; solution (2) as a freshly prepared 0.05 per cent w/v solution of *tetracycline hydrochloride R.S.* in *methyl alcohol*.

(B) To a quantity of the contents of the capsules equivalent to 10 mg of Tetracycline Hydrochloride, add 20 ml of warm *alcohol*, allow to stand for fifteen minutes, filter and evaporate the filtrate to dryness on a water-bath. The residue complies with **Identification** tests (B) and (C) described under Tetracycline Hydrochloride.

Light-absorbing impurities : Dissolve the contents of five capsules as completely as possible in sufficient *0.01N hydrochloric acid* to produce a solution containing the equivalent of 0.125 per cent w/v of Tetracycline Hydrochloride and filter through paper, rejecting the first 20 ml of the filtrate. *Extinction* of a 1-cm layer of the filtrate at 430 nm, measured within one hour of introducing the contents to the solvent, not greater than 0.62, Appendix 5.15 A.

Other requirements : Comply with the requirements stated under Capsules.

Loss on drying : Not more than 3.0 per cent, determined on 1.0 g of the contents of the capsules by drying "in vacuo at 60°" for three hours, Appendix 5.8.

Assay : Weigh accurately a quantity of the mixed contents of 20 capsules equivalent to 0.25 g of Tetracycline Hydrochloride, add 500.0 ml of *0.1N hydrochloric acid*. Carry out the *microbiological assay of antibiotics, Method A* or *B*, Appendix 4.1. Calculate the content of tetracycline hydrochloride in the average weight of the capsules.

Storage : Store in tightly-closed containers in a cool place.

Labelling : The label on the container states (1) the date after which the capsules are not intended to be used; (2) the storage conditions.

Tetracycline Injection

Tetracycline Hydrochloride Injection

Category : Antibacterial; anti-amoebic; anti-rickettsial.

Dose : Tetracycline Hydrochloride. By intravenous injection, 1 to 2 g daily in a concentration not exceeding 0.1 per cent w/v.

By intramuscular injection, 250 mg to 800 mg daily.

Usual strengths : 100 mg; 250 mg; 500 mg.

Standards : Tetracycline Injection is a sterile solution of Tetracycline Hydrochloride in Water for Injection. It is prepared by dissolving the contents of a sealed container in the requisite amount of Water for Injection; the sealed container also contains a suitable buffering or stabilising agent.

Content of tetracycline hydrochloride : Determine the weight of the contents of each of ten containers as described in the test for **Uniformity of weight** under Injections. Mix the contents of the ten containers and carry out the *microbiological assay of antibiotics, Method A* or *B*, Appendix 4.1. From the result of the assay calculate the proportionate amount of tetracycline hydrochloride in each container. This amount is not less than 90.0 per

cent and not more than 115.0 per cent of the amount stated on the label.

Other requirements : Complies with the requirements stated under Injections.

The contents of the sealed container comply with the following requirements:

Description : Pale-yellow, crystalline powder.

Identification : (A) Comply with **Identification** test (A) described under Oxytetracycline Hydrochloride, solution (1) being freshly prepared by shaking a quantity equivalent to 10 mg of Tetracycline Hydrochloride with 20 ml of *methyl alcohol*, centrifuging if necessary, and using the clear liquid; solution (2) is a freshly prepared 0.05 per cent w/v solution of *tetracycline hydrochloride R.S.* in *methyl alcohol.*

(B) Comply with the **Identification** tests (B) and (C) described under Tetracycline Hydrochloride.

Clarity and colour of solution : A 10 per cent w/v solution is clear and yellow.

pH : Between 2.0 and 3.0, determined in a 10 per cent w/v solution, Appendix 5.10.

Light-absorbing impurities : *Extinction* at 430 nm of a solution in *0.01N hydrochloric acid* containing the equivalent of 0.2 per cent w/v of Tetracycline Hydrochloride, measured within one hour of introducing the substance to the solvent, not greater than 0.50, Appendix 5.15 A.

Undue toxicity : Comply with the test described under Oxytetracycline, the dose being 0.5 ml of a solution containing 2 mg of Tetracycline Hydrochloride per ml.

Histamine-like substances : Comply with the test for *histamine-like substances*, Appendix 2.35, using a solution containing 5 mg of Tetracycline Hydrochloride per ml.

Pyrogens : Comply with the **Test for pyrogens**, Appendix 2.36, using a volume not exceeding 5 ml containing not less than 5 mg of Tetracycline Hydrochloride per kg of the rabbit's weight, dissolved in *water for injection.*

Sterility : Comply with the *tests for sterility*, Appendix 4.6.

Storage : Store in light-resistant containers. The constituted solution should be used within three days when stored in a cold place.

Labelling : The label on the sealed container states (1) the weight of Tetracycline Hydrochloride contained in it; (2) the name of the buffering or stabilising agent; (3) that the contents are to be used for intravenous injection only, in a well-diluted solution; (4) the date after which the contents are not intended to be used; (5) the storage conditions.

Tetracycline Tablets

Tetracycline Hydrochloride Tablets

Category : Antibacterial.

Dose : Tetracycline Hydrochloride. For adults, 1 to 3 g daily, in divided doses; for children, 10 to 30 mg per kg of body weight daily, in divided doses.

Usual strength : 250 mg.

Standards : Tetracycline Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of Tetracycline Hydrochloride, $C_{22}H_{24}N_2O_8$,HCl. The tablets may be coated.

Identification : (A) Comply with **Identification** test (A) described under Doxycycline Hydrochloride, solution (1) being freshly prepared by extracting a quantity of the powdered tablets equivalent to 10 mg of Tetracycline Hydrochloride with 20 ml of *methyl alcohol*, centrifuging, and using the supernatant liquid; solution (2) is a freshly prepared 0.05 per cent w/v solution of *tetracycline hydrochloride R.S.*, in *methyl alcohol.*

(B) Extract a quantity of the powdered tablets equivalent to 10 mg of Tetracycline Hydrochloride with 25 ml of *alcohol* for fifteen minutes, filter, and evaporate the filtrate to dryness on a water-bath. The residue complies with **Identification** tests (B) and (C) described under Tetracycline Hydrochloride.

Light-absorbing impurities : Wash five tablets with *water* until any coloured coating is removed, dry with filter paper, and powder. Dissolve the powder as completely as possible in sufficient *0.01N hydrochloric acid* to produce a solution containing the equivalent of 0.125 per cent w/v of Tetracycline Hydrochloride and filter through paper, rejecting the first 20 ml of the filtrate. *Extinction* of a 1-cm layer of the filtrate at 430 nm, measured within one hour of introduction of the powder to the solvent, not greater than 0.62, Appendix 5.15 A.

Disintegration : Maximum time, thirty minutes, Appendix 5.6.1.

Dissolution : Comply with the *dissolution test for tablets and capsules*, Appendix 5.7, using as the medium 1000 ml of *0.1N hydrochloric acid*, and placing one tablet in the basket for each test. Withdraw a sample of 10 ml of the medium and filter. Measure the *extinction* of the filtered solution, suitably diluted if necessary, at the maximum at about 353 nm, Appendix 5.15 A. Calculate the total content of $C_{22}H_{24}N_2O_8$,HCl, in the medium, taking 320 as the value of E(1 per cent, 1-cm) at the maximum at about 353 nm.

Other requirements : Comply with the requirements stated under Tablets.

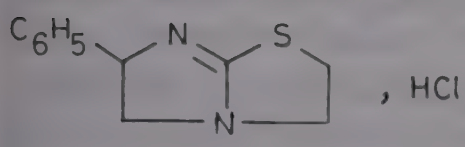
Loss on drying : Not more than 3.0 per cent, determined on 1.0 g of the powdered tablets by drying "in vacuo at 60°" for three hours, Appendix 5.8.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets equivalent to 0.25 g of Tetracycline Hydrochloride, triturate with several quantities of *0.1N hydrochloric acid*, transfer completely to a 1000-ml graduated flask and make to volume with *0.1N hydrochloric acid*. Shake, filter, and carry out the *microbiological assay of antibiotics, Method A* or *B*, Appendix 4.1.

Storage : Store in tightly-closed containers.

Labelling : The label on the container states (1) the date after which the tablets are not intended to be used; (2) the storage conditions.

Tetramisole Hydrochloride



$C_{11}H_{12}N_2S,HCl$ Mol. Wt. 240.75

Category : Anthelmintic.

Dose : Oral, 2.5 to 5 mg per kg body weight.

Description : White to pale cream-coloured crystalline powder; odourless or almost odourless.

Solubility : Soluble in *water* and in *methyl alcohol*, very slightly soluble in *solvent ether*.

Standards : Tetramisole Hydrochloride is the hydrochloride of (±)-2,3,5,6-tetrahydro-6-phenylimidazo(2,1-b)thiazole. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{11}H_{12}N_2S,HCl$, calculated with reference to the dried substance.

Identification : (A) Dissolve 0.5 g in 20 ml of *water* and 6 ml of *N sodium hydroxide*. Extract with 20 ml of *methylene chloride*, discard the aqueous layer and wash the methylene chloride extract with 10 ml of *water*. Shake with *anhydrous sodium sulphate*, filter, evaporate the filtrate at room temperature, and dry "in vacuo at a temperature not exceeding 40°". The residue obtained melts at about 91°, Appendix 5.11.

(B) A 5.0 per cent w/v solution is optically inactive, Appendix 5.12.

(C) A solution (1 in 20) gives the reactions of *chlorides*, Appendix 3.1.

Light absorption : *Extinction* of a 1-cm layer of a 0.1 per cent w/v solution in *0.2N methanolic hydrochloric acid* at 310 nm, not more than 0.2, Appendix 5.15 A.

2,3-dihydro-6-phenylimidazo(2,1-b)-thiazole hydrochloride : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 45 volumes of *toluene*, 8 volumes of *methyl alcohol* and 4 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in *methyl alcohol* containing (1) 5.0 per cent w/v of the substance being examined, and (2) 0.025 per cent of w/v of *2,3-dihydro-6-phenylimidazo(2,1-b)-thiazole hydrochloride R.S.* After removal of the plate allow it to dry in air and spray with *potassium iodoplatinate solution*. The spot in the chromatogram obtained with solution (1) is not more intense than any corresponding spot in the chromatogram obtained with solution (2)

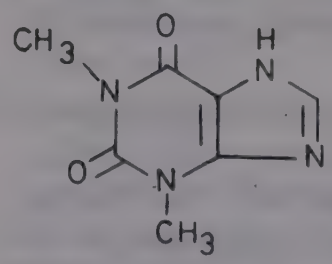
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and dissolve in 50 ml of *glacial acetic acid*. Add 10 ml of *mercuric acetate solution* and titrate with *0.1N perchloric acid*, using *1-naphtholbenzein solution* as indicator. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02408 g of $C_{11}H_{22}N_2S,HCl$.

Storage : Store in well-closed, light-resistant containers.

Theophylline



$C_7H_8N_4O_2$ Mol. Wt. 180.17 (anhydrous)

$C_7H_8N_4O_2,H_2O$ Mol. Wt. 198.18 (hydrated)

Category : Pharmaceutical necessity for Aminophylline Injection.

Description : White, crystalline or amorphous powder; odourless; taste, bitter.

Solubility : Slightly soluble in *water*, in *alcohol* and in *chloroform*; very slightly soluble in *solvent ether*; freely soluble in solutions of the alkali hydroxides, in *strong ammonia solution* and in mineral acids.

Standards : Theophylline is 1, 3-dimethyl - 2, 6-(1*H*,3*H*)-purinedione. It contains one molecule of water of hydration or is anhydrous. It contains not

less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_7H_8N_4O_2$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** tests (B) and (C) described under Aminophylline.

(B) Dissolve 0.2 g in 5 ml of *potassium hydroxide solution* or in 5 ml of *strong ammonia solution*; a clear solution is produced.

Melting range : Between 269° and 274°, Appendix 5.11.

Light absorption : *Extinction* of a 1-cm layer of a 0.001 per cent w/v solution in *0.1N hydrochloric acid* at 270 nm not less than 0.53, calculated with reference to the dried substance, Appendix 5.15 A.

Acidity : Dissolve 0.5 g in 75 ml of *water*, add one drop of *methyl red solution* and titrate with *0.02N sodium hydroxide*; not more than 1.0 ml is required.

Clarity of solution : Dissolve 1.0 g in 10 ml of boiling *water*. The hot solution is clear or at most very slightly opalescent.

Theobromine : To 1.0 g add 10 ml of *6N ammonia* and shake; the solution is clear.

Caffeine : To 1.0 g add 10 ml of *6N ammonia*, shake, add 30 ml of *water*, 15 ml of *2N sodium hydroxide* and 50 ml of *chloroform*. Shake, separate the chloroform layer, and dry it over *anhydrous sodium sulphate*. Filter, evaporate 25 ml of the filtrate and dry the residue in a desiccator; the residue weighs not more than 5 mg.

Alkaloids : To 0.5 g add 5 ml of *water* and 1 ml of *dilute sulphuric acid*, shake vigorously and filter. The filtrate, to which five drops of *potassium mercuri-iodide solution* have been added, remains clear for not less than five minutes.

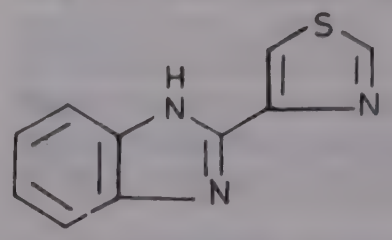
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent (for the anhydrous form) and not more than 9.5 per cent (for the hydrated form), determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.25 g, add 50 ml of *water* and gently warm the mixture on a water-bath until complete solution is effected. Cool, add 20.0 ml of *0.1N silver nitrate* and 1.0 ml of *bromothymol blue solution*, and titrate with *0.1N sodium hydroxide* to a blue end-point. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01802 g of $C_7H_8N_4O_2$.

Storage : Store in well-closed containers.

Thiabendazole



$C_{10}H_7N_3S$ Mol. Wt. 201.25

Category : Anthelmintic.

Dose : 1.5 g, twice daily for two or three days, in the treatment of nematode infestation.

Description : White or almost white powder; odourless or almost odourless.

Solubility : Slightly soluble in *acetone*, and in *alcohol*; very slightly soluble in *chloroform*, and in *solvent ether*; practically insoluble in *water*.

Standards : Thiabendazole is 2-(thiazol-4-yl)benzimidazole. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{10}H_7N_3S$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to those in the spectrum of *thiabendazole R.S.*, Appendix 5.15 B.

(B) Dissolve 5 mg in 5 ml of *0.1N hydrochloric acid*, add 3 mg of *p-phenylenediamine dihydrochloride*, shake until dissolved, add 0.1 g of *zinc powder*, mix, and allow to stand for two minutes; the odour of hydrogen sulphide is produced. Add 10 ml of *ferric ammonium sulphate solution*; a deep blue or blue-violet colour is produced.

CAUTION — p-phenylenediamine dihydrochloride may cause dermatitis. It should be handled with care and contact with skin should be avoided.

(C) It melts at about 300°, Appendix 5.11.

(D) The light absorption in the range 230 to 350 nm of a 1-cm layer of a 0.0004 w/v solution in *0.1N hydrochloric acid* exhibits two maxima at 243 and 302 nm, Appendix 5.15 A; *extinction* at 243 nm, about 0.24 and at 302 nm, about 0.49, Appendix 5.15 A.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method B, Appendix 3.2.4.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel GF 254* as the coating substance and a mixture of 50 volumes of *toluene*, 20 volumes of *glacial acetic acid*, 8 volumes of *acetone* and 2 volumes of *water* as the mobile phase. Apply separately to the plate 10 µl of each two solutions in *methyl alcohol* containing (1) 1.0 per cent w/v of the substance being examined and (2) 0.015

per cent w/v of the substance being examined. After removal of the plate allow it to dry in air and examine under an ultra-violet lamp having a maximum output at about 254 nm. Any spot in the chromatogram obtained with solution (1), other than the principal spot, is not more intense than the spot in the chromatogram obtained with solution (2).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.16 g of Thiabendazole and dissolve in 10 ml of *glacial acetic acid*. Add 50 ml of *acetic anhydride*, 1 ml of *mercuric acetate solution* and 2 drops of *crystal-violet solution* and titrate with *0.1N perchloric acid* to a blue-green end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02013 g of $C_{10}H_7N_3S$.

Storage : Store in well-closed containers.

Thiabendazole Tablets

Category : Anthelmintic.

Dose : Thiabendazole, 1.5 g twice daily for two or three days in the treatment of nematode infestation.

Usual strength : 0.5 g.

Standards : Thiabendazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Thiabendazole, $C_{10}H_7N_3S$. The tablets may contain suitable flavouring and sweetening agents.

Identification : Complies with **Identification** test (A) described under Thiabendazole, using a quantity of the powdered tablets equivalent to 20 mg of Thiabendazole.

Disintegration : The requirement for Disintegration, does not apply to Thiabendazole Tablets.

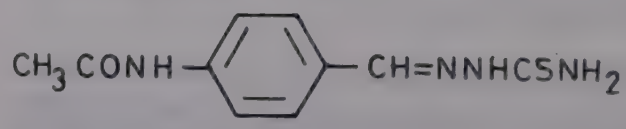
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder twenty tablets. To an accurately weighed quantity of the powdered tablets, equivalent to about 0.1 g of Thiabendazole, add 75 ml of *0.1N hydrochloric acid*, warm on a water-bath for fifteen minutes with occasional shaking. Cool, dilute to 100.0 ml with *0.1N hydrochloric acid*, and filter rejecting the first 20 ml of the filtrate. Dilute 5.0 ml of the clear filtrate to 1000.0 ml with *0.1N hydrochloric acid* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 302 nm, Appendix 5.15 A. Calculate the contents of $C_{10}H_7N_3S$ taking 1230 as the value of E(1 per cent, 1-cm) at the maximum at about 302 nm.

Storage : Store in well-closed, light-resistant containers.

Labelling : The label on the container states "chew the tablets before swallowing"

Thiacetazone



$C_{10}H_{12}N_4OS$ Mol. Wt. 236.29

Category : Tuberculostatic.

Dose : 0.15 g daily.

Description : Pale-yellow crystals or crystalline powder; almost odourless.

Solubility : Very slightly soluble in *water*, slightly soluble in *alcohol*; soluble in *propylene glycol*.

Standards : Thiacetazone is *p*-acetamidobenzaldehyde thiosemicarbazone. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{10}H_{12}N_4OS$, calculated with reference to the dried substance.

Identification : (A) Boil 10 mg with 5 ml of *N hydrochloric acid* for three minutes, cool and add sufficient *water* to produce 200 ml. Mix 5 ml of this solution with 0.25 ml of *sodium nitrite solution*, and add the mixture to 0.5 ml of *β-naphthol solution*; a red colour is produced.

(B) Dissolve, with the aid of heat, 10 mg in 1 ml of *6N sodium hydroxide*, add 0.25 ml of *lead acetate solution*, and boil for one minute; a black precipitate is produced.

(C) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of 0.0003 per cent w/v solution in *alcohol* exhibits a maximum only at about 328 nm; *extinction* at 328 nm, about 0.55, Appendix 5.15 A.

(D) It melts at about 220°, with decomposition, Appendix 5.11.

Thiosemicarbazide : To 2 g, finely powdered, add sufficient *water* to produce 50 ml, shake, allow to stand for one hour with occasional shaking, and filter, discarding the first portion of the filtrate; acidify 25 ml of the clear filtrate with *dilute sulphuric acid*, add 0.1 ml of *o-phenanthroline-ferrous complex solution*, and titrate with *0.1N ceric ammonium sulphate* to a blue end-point which persists for one minute; not more than 0.8 ml is required.

***p*-acetamidobenzalazine** : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using a suitable silica gel with a binder and fluorescent additive as

the coating substance and *ethyl acetate* as the mobile phase. Apply separately to the plate 5 µl of solution (1) and 10 µl of solution (2) prepared as follows:

For solution (1), dissolve 0.4 g of the substance being examined in sufficient *methyl alcohol* to produce 100 ml.

Solution (2) is prepared by dissolving with the aid of heat 0.016 g of *p-acetamidobenzalazine R.S.*, in 150 ml of *methyl alcohol*, cooling and diluting to 200 ml with *methyl alcohol* and further diluting 5 ml of this solution to 100 ml with *methyl alcohol*.

After removal of the plate, allow it to dry in a current of air, spray with *2N nitric acid*, and within two minutes, examine the plate under ultra-violet radiation. Any spot in the chromatogram obtained with solution (1) other than the principal spot is not more intense than the spot in the chromatogram obtained with solution (2).

Heavy metals : Not more than 10 parts per million, determined by *Method B* on 2.0 g, Appendix 3.2.4.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.1 g, dissolve in 60 ml of *methyl alcohol* by heating at 60° on a water-bath, slowly add 20 ml of hot *methanolic silver nitrate solution*, maintain the solution at 60° until the precipitate coagulates and leaves a clear supernatant liquid; cool, filter through a sintered-glass crucible (porosity 4), wash the residue with *methyl alcohol* until the washings are free from silver nitrate, and dry to constant weight at 105°. Each g of residue is equivalent to 0.4606 g of $C_{10}H_{12}N_4OS$.

Storage : Store in well-closed, light-resistant containers.

Thiacetazone and Isoniazid Tablets

Category : Antibacterial (tuberculostatic).

Dose : Thiacetazone, 150 mg and Isoniazid, 300 mg; daily, in single or divided doses.

Usual strengths : Thiacetazone, 37.5 mg and Isoniazid, 75 mg; Thiacetazone, 75 mg and Isoniazid, 150 mg; Thiacetazone, 150 mg and Isoniazid, 300 mg.

Standards : Thiacetazone and Isoniazid Tablets contain one part by weight of Thiacetazone and two parts by weight of Isoniazid. They contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amounts of Thiacetazone, $C_{10}H_{12}N_4OS$, and Isoniazid, $C_6H_7N_3O_2$.

Identification : (A) Extract a quantity of the powdered tablets equivalent to about 30 mg of Thiacetazone, add 70 ml of *alcohol* and heat on a water-bath for fifteen minutes with intermittent shaking. Cool, make upto 100 ml with *alcohol* and filter. Dilute 1 ml to 100 ml with *alcohol*. The resulting solution complies with **Identification** test (C) described under Thiacetazone.

(B) Shake a quantity of the powdered tablets equivalent to about 1 mg of Isoniazid, with 50 ml of *alcohol* and filter; 5 ml of the filtrate complies with **Identification** test (C) described under Isoniazid.

Disintegration : Maximum time, thirty minutes, Appendix 5.6.1.

Other requirements : Comply with the requirements stated under Tablets.

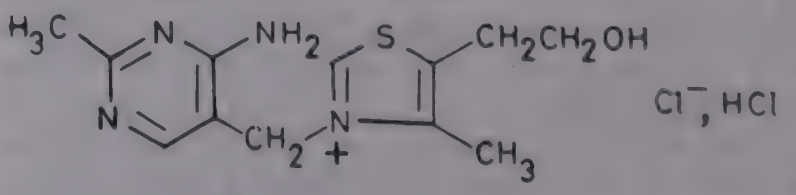
Assay : *For Thiacetazone* — Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 30 mg of Thiacetazone, add 70 ml of *alcohol* and heat on a water-bath for fifteen minutes with intermittent shaking. Cool, make upto 100.0 ml with *alcohol* and filter. Dilute 1.0 ml of the filtrate to 100.0 ml with *alcohol*. Measure the *extinction* of a 1-cm layer of the filtrate at the maximum at about 328 nm using *alcohol* as the blank, Appendix 5.15 A. Calculate the content of $C_{10}H_{12}N_4OS$ from the *extinction* obtained by repeating the operation using a 0.0003 per cent w/v solution of *thiacetazone R.S.* and from the declared content of $C_{10}H_{12}N_4OS$ in the *thiacetazone R.S.*

For Isoniazid—Weigh accurately a quantity of the powder equivalent to about 0.4 g of Isoniazid, dissolve as completely as possible in 100 ml of *water* and filter. Wash the residue with *water*, combine the filtrate and washings and dilute to 250.0 ml with *water*. Transfer 25.0 ml of the resulting solution to a glass-stoppered flask and carry out the **Assay** described under Isoniazid, beginning at the words "add 25 ml of *0.1N bromine*.....". Perform a blank determination by repeating the operation omitting the substance being examined but including a quantity of Thiacetazone equivalent to the amount present in the weight of sample taken for the assay of isoniazid.

Storage : Store in light-resistant containers.

Thiamine Hydrochloride

Thiamine Chloride, Aneurine Hydrochloride, Vitamin B_1



$C_{12}H_{17}ClN_4OS,HCl$ Mol. Wt. 337.27

Category : Vitamin B_1 (enzyme cofactor).

Dose : Prophylactic, 2 to 5 mg once a day; therapeutic, 25 to 100 mg daily.

Description : White or almost white crystalline powder or colourless crystals; odour, slight and characteristic; taste, bitter.

Solubility : Freely soluble in *water*; slightly soluble in *alcohol*; practically insoluble in *solvent ether* and in *acetone*.

Standards : Thiamine Hydrochloride is the hydrochloride of 3-[(4-amino-2-methylpyrimidin-5-yl)-methyl] - 5 - (2-hydroxyethyl) - 4 - methylthiazolium chloride. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{12}H_{17}ClN_4OS,HCl$, calculated with reference to the dried substance.

Identification : (A) An aqueous solution yields a white precipitate with *mercuric chloride solution* and a red-brown precipitate with *iodine solution*.

(B) Dissolve about 10 mg in 10 ml of *2N sodium hydroxide*, add 10 ml of *potassium ferricyanide solution* and 5 ml of *n-butyl alcohol*, shake for two minutes, and allow to separate; the alcoholic layer shows an intense blue fluorescence which disappears on acidification and reappears on making alkaline.

(C) The light absorption, in the range 230 to 350 nm, of a 0.002 per cent w/v solution exhibits maxima at 233 nm and at 267 nm; *extinction* at 233 nm, about 0.68 and at 267 nm, about 0.52, Appendix 5.15 A.

(D) A solution (1 in 20) gives the reactions of *chlorides*, Appendix 3.1.

Clarity and colour of solution : A 10.0 per cent w/v solution is clear and not more coloured than a solution containing 0.15 ml of *0.1N potassium dichromate* in sufficient *water* to produce 100.0 ml.

pH : Between 2.7 and 3.3, determined in a 2.5 per cent w/v solution, Appendix 5.10.

Nitrate : To 2 ml of a 2.0 per cent w/v solution add 2 ml of *sulphuric acid*, cool and superimpose 2 ml of *ferrous sulphate solution*; no brown ring is produced at the junction of the layers.

Heavy metals : Not more than 20 parts per million, determined by Method A on a solution prepared by dissolving 1.0 g in 20 ml of *water*, Appendix 3.2.4.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 50 mg and dissolve in 50 ml of *water*, add 2 ml of *hydrochloric acid* and heat to boiling. To the boiling solution, rapidly add, drop by drop, 4 ml of freshly filtered *silicotungstic acid solution*, boil for four minutes, filter through a sintered glass crucible, and wash with a boiling mixture of 1 volume of *hydrochloric acid* and 19 volumes of *water*, and then with 10 ml of *water* and two quantities, each of 5 ml of *acetone*. Dry the residue to constant weight at 105°. Each g of residue is equivalent to 0.1929 g of $C_{12}H_{17}ClN_4OS,HCl$.

Storage : Store in tightly-closed, light-resistant, non-metallic containers.

Thiamine Hydrochloride Injection

Aneurine Hydrochloride Injection; Vitamin B_1 Injection

Category : Vitamin B_1 (enzyme cofactor).

Dose : Thiamine Hydrochloride. By subcutaneous or intramuscular injection, 25 to 100 mg.

Usual strength : 100 mg per ml.

Description : Clear, colourless or almost colourless solution.

Standards : Thiamine Hydrochloride Injection is a sterile solution of Thiamine Hydrochloride in Water for Injection. It contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of $C_{12}H_{18}Cl_2N_4OS$.

Identification : (A) Complies with **Identification** tests (A) and (D) described under Thiamine Hydrochloride.

(B) To a volume equivalent to 20 mg of Thiamine Hydrochloride, diluted, if necessary, to 10 ml with *water*, add 2 ml of *dilute acetic acid* and 1.6 ml of *N sodium hydroxide*, heat in a water-bath for thirty minutes, and cool. Add 5 ml of *sodium hydroxide solution*, 10 ml of *potassium ferricyanide solution*, and 10 ml of *n-butyl alcohol* and shake vigorously for two minutes. The alcohol layer shows an intense light blue fluorescence, especially on exposure to ultra-violet light. Repeat the test adding 0.9 ml of *N sodium hydroxide* and 0.2 g of *sodium sulphite* instead of 1.6 ml of *N sodium hydroxide*; no flourescence is produced.

pH : Between 2.5 and 4.5, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

Assay : Measure accurately a volume equivalent to about 25 mg of Thiamine Hydrochloride, dilute to 50 ml with *water*, add 2 ml of *hydrochloric acid* and heat to boiling. To the boiling solution, rapidly add drop by drop 2 ml of *silicotungstic acid solution* and complete the **Assay** described undet Thiamine Hydrochloride, beginning at the words "boil for four minutes.....".

Storage : Store in single-dose or multiple-dose, light-resistant containers.

Thiamine Hydrochloride Tablets

Aneurine Hydrochloride Tablets; Vitamin B_1 Tablets

Category : Vitamin B_1 (enzyme cofactor).

Dose : Thiamine Hydrochloride, prophylactic 5 to 10 mg daily; therapeutic 10 to 35 mg three times daily.

Usual strengths : 5 mg; 10 mg; 50 mg.

Standards : Thiamine Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Thiamine Hydrochloride, $C_{12}H_{18}Cl_2N_4OS$.

Identification : (A) Dissolve a quantity of the powdered tablets equivalent to 20 mg of Thiamine Hydrochloride as completely as possible in 10 ml of *water* and filter. The filtrate complies with **Identification** tests (A) and (B) described under Thiamine Hydrochloride.

(B) The powdered tablets give the reactions of *chlorides*, Appendix 3.1.

Uniformity of content *(for tablets containing 5 mg and 10 mg)*: Finely crush one tablet, add 20 ml of *alcohol*, stir the mixture for thirty minutes and centrifuge. Repeat the extraction with three further quantities, each of 15 ml, of *alcohol*. Combine the extracts, add sufficient *alcohol* to produce 100.0 ml and mix. Dilute a suitable volume of the resulting solution equivalent to 1 mg of Thiamine Hydrochloride with sufficient *alcohol* to produce 100.0 ml. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 233 nm, using *alcohol* as the blank, Appendix 5.15 A. Calculate the content of $C_{12}H_{18}Cl_2N_4OS$, taking 380 as the value of E(1 per cent, 1-cm) at the maximum at about 233 nm.

Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 and 110 per cent of the average except that for one tablet the content may be between 85 and 115 per cent of the average.

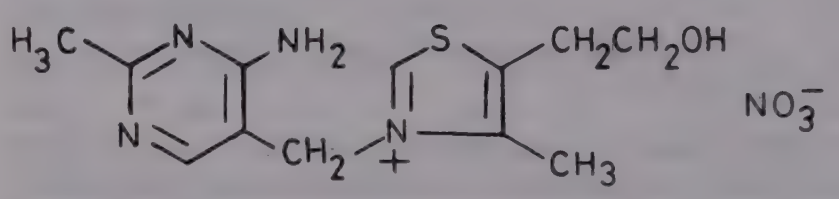
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets or more if necessary. Weigh accurately a quantity of the powder equivalent to 50 mg of Thiamine Hydrochloride, add 60 ml of a mixture of 250 ml of *water* and 10 ml of *hydrochloric acid* and allow to stand for one hour, shaking occasionally. Filter, wash the residue with several quantities of the mixture of *hydrochloric acid* and *water*, add to the combined filtrate and washings sufficient *water* to produce 100.0 ml and mix. Pipette 50 ml into a beaker, heat rapidly to boiling, add drop by drop to the boiling solution, 2 ml of freshly filtered *silicotungstic acid solution* and complete the **Assay** described under Thiamine Hydrochloride, beginning at the words "boil for four minutes.....".

Storage : Store in tightly-closed, light-resistant containers.

Thiamine Mononitrate

Thiamine Nitrate



$C_{12}H_{17}N_5O_4S$ Mol. Wt. 327.36

Category : Vitamin B_1.

Dose : Prophylactic, 2 to 5 mg daily; therapeutic, 25 to 100 mg daily.

Description : White crystals or crystalline powder; odour, slight and characteristic; taste, slightly bitter.

Solubility : Sparingly soluble in *water*; slightly soluble in *alcohol* and in *chloroform*.

Standards : Thiamine Mononitrate is 3-[(4-amino-2-methylpyrimidin-5-yl) methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{12}H_{17}N_5O_4S$, calculated with reference to the dried substance.

Identification : (A) Complies with **Identification** tests (A) and (B) described under Thiamine Hydrochloride.

(B) The light absorption, in the range 230 to 350 nm, of a 0.002 per cent w/v solution exhibits maxima at 233 nm and at 267 nm; *extinction* at 233 nm, about 0.69 and at 267 nm, about 0.53, Appendix 5.15 A.

(C) To 2 ml of a 2 per cent w/v solution add 2 ml of *sulphuric acid*, cool and superimpose 2 ml of *ferrous sulphate solution*; a brown ring is produced at the junction of the two liquids.

pH : Between 6.0 and 7.5, determined in a 2.0 per cent w/v solution, Appendix 5.10.

Chloride : 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Heavy metals : Not more than 20 parts per million, determined by Method A on 1.0 g dissolved in 20 ml of *water*, Appendix 3.2.4.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

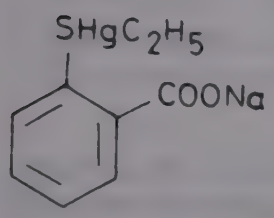
Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for two hours, Appendix 5.8.

Assay : Carry out the **Assay** described under Thiamine Hydrochloride. Each g of residue is equivalent to 0.1879 g of $C_{12}H_{17}N_5O_4S$.

Storage : Store in tightly-closed, light-resistant containers.

Thiomersal

Thimerosal



$C_9H_9HgNaO_2S$ Mol. Wt. 404.81

Category : Pharmaceutical aid (antiseptic, preservative).

Description : Light, cream-coloured, crystalline powder; odour, slight and characteristic.

Solubility : Freely soluble in *water* and in *alcohol*; practically insoluble in *solvent ether*.

Standards : Thiomersal is the sodium salt of (2-carboxyphenylthio) ethylmercury. It contains not less than 97.0 per cent and not more than the equivalent of 101.0 per cent of $C_9H_9HgNaO_2S$, calculated with reference to the dried substance.

Identification : (A) Dissolve 0.5 g in 10 ml of *water* and add 2 ml of *dilute hydrochloric acid*; a white precipitate is produced which, after washing with *water* and drying "in vacuo" over *phosphorus pentoxide*, melts at about 110°, Appendix 5.11.

(B) Dissolve 0.1 g in 10 ml of *water* and add 2 ml of *silver nitrate solution*; a white precipitate is produced.

pH : Between 6.0 and 8.0, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Ether-soluble substances : Not more than 0.8 per cent, determined by the following method: Shake 0.50 g with 20 ml of *solvent ether* for ten minutes, filter, and evaporate the filtrate to dryness. Dry the residue over *phosphorus pentoxide* "in vacuo" for twenty-four hours.

Mercuric salts : Dissolve 0.1 g in 10 ml of *water* and add an equal volume of *ammonium sulphide solution*: a white precipitate is produced. Allow to stand for thirty minutes in the dark; no blackening occurs.

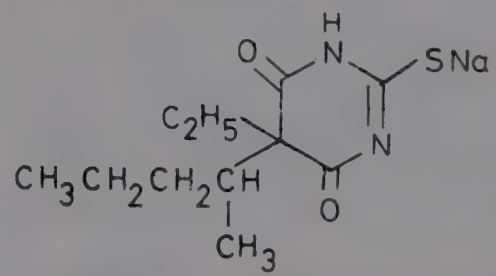
Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying "in vacuo" for twenty-four hours, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and transfer to a 100 ml long-necked flask, add 5 ml of *sulphuric acid* and heat gently until charring occurs; continue to heat and add *strong hydrogen peroxide solution* dropwise, until the mixture is colourless. Dilute with *water*, cool, and titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N ammonium thiocyanate* is equivalent to 0.02024 g of $C_9H_9HgNaO_2S$.

Storage : Store in tightly closed, light-resistant containers.

Thiopentone Sodium

Thiopental Sodium



$C_{11}H_{17}N_2NaO_2S$ Mol. Wt. 264.32

Category : Anticonvulsant; general anaesthetic.

Dose : By intravenous injection, 0.1 to 0.5 g; by rectal injection, 40 mg per kg of body weight. Maximum dose, 2 g.

Description : Yellowish-white powder; odour, faint and resembling garlic; taste, bitter; hygroscopic.

Solubility : Soluble in *water*; partially soluble in *alcohol*; practically insoluble in *benzene* and in *solvent ether*.

Standards : Thiopentone Sodium is a mixture of sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate and anhydrous sodium carbonate. It contains the equivalent of not less than 84.0 per cent and not more than 87.0 per cent of thiopentone, $C_{11}H_{18}N_2O_2S$, and not less than 10.0 per cent and not more than 11.0 per cent of sodium, Na, both calculated with reference to the dried substance.

Identification : (A) Acidify 5 ml of a 5.0 per cent w/v solution with *dilute acetic acid* and filter. Wash the

precipitate with *water*, recrystallise from *water* and dry at 70°; the crystals melt at about 160°, Appendix 5.11.

(B) Dissolve 1 mg of the crystals obtained in **Identification** test (A) in 1 ml of *0.1N sodium hydroxide*. Add about 1 mg of *sodium nitroprusside* and after fifteen minutes, 1 ml of *dilute hydrochloric acid*; a reddish-violet colour is produced.

(C) Evaporate the filtrate obtained in **Identification** test (A). The residue gives the reactions of *sodium*, Appendix 3.1.

Chloride : Dissolve 0.25 g in 20 ml of *water*, add 5 ml of *dilute nitric acid* and extract with three quantities, each of 25 ml, of *solvent ether*; the aqueous layer complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : Dissolve 0.25 g in 25 ml of *water*, add 3 ml of *dilute nitric acid* and filter. To 10 ml of the filtrate add 10 ml of *water* and 1 ml of *barium chloride solution*; not more than a slight turbidity is produced.

Loss on drying : Not more than 2.5 per cent, determined on 0.5 g by drying "in vacuo at 100°" for four hours, Appendix 5.8.

Assay : *For Sodium* - Weigh accurately about 0.6 g and dissolve in 20 ml of *water*, add 1 drop of *methyl red solution* and titrate with *0.1N sulphuric acid* until the yellow colour changes to pink; boil gently for one or two minutes, cool, and if necessary, continue the titration with *0.1N sulphuric acid* until the pink colour is restored. Each ml of *0.1N sulphuric acid* is equivalent to 0.002299 g of Na.

For Thiopentone—To the liquid from the **Assay** *for sodium* add a further 5 ml of *0.1N sulphuric acid* and extract with successive quantities of 25, 25, 20, 15, 15 and 10 ml of *chloroform*, washing each extract with the same 5 ml of *water*; remove the chloroform from the mixed extracts by evaporation and dry the residue to constant weight at 100° to 105°.

Storage : Store in tightly-closed containers, protected from moisture.

Thiopentone Injection

Thiopental Sodium for Injection

Category : Anticonvulsant; general anaesthetic.

Dose : Thiopentone Sodium. By intravenous injection, 0.1 to 0.5 g.

Usual strengths : 0.25 g; 0.5 g.

Standards : Thiopentone Injection is a sterile solution of Thiopentone Sodium in Water for Injection. It is prepared by dissolving the contents of a sealed container in the requisite amount of Water for Injection immediately before use.

Content of thiopentone, $C_{11}H_{18}N_2O_2S$: Determine the weight of the contents of each of ten containers as described in the test for **Uniformity of weight** under Injections. The weight of the contents of each container does not deviate from the weight stated on the label by more than 10 per cent except that in one container the weight may deviate by not more than 15 per cent.

Other requirements : Complies with the requirements stated under Injections.

The contents of the sealed container comply with the following requirements:

Description : Yellowish-white powder; hygroscopic.

Identification : Comply with **Identification** tests (A) to (C) described under Thiopentone Sodium.

Appearance of reconstituted solution : Dissolve 0.8 g of the mixed contents of ten containers in 10 ml of *carbon dioxide-free water*; after one minute the solution is clear and free from undissolved particles.

pH : Between 10.2 and 11.2, determined in the solution prepared in the test for **Appearance of reconstituted solution**, Appendix 5.10.

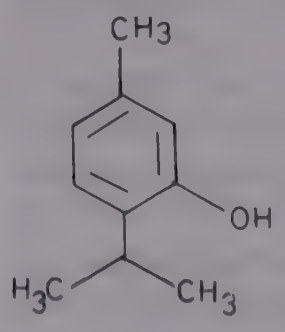
Loss on drying : Not more than 2.5 per cent, determined on 1.0 g by drying "in vacuo at 100°" for four hours, Appendix 5.8.

Assay : Carry out the **Assay**, described under Thiopentone Sodium, using the mixed contents of ten containers.

Storage : Store in single-dose containers. The Injection should be freshly prepared and used within twenty-four hours.

Labelling : The label on the container states the weight of Thiopentone Sodium contained in it.

Thymol



$C_{10}H_{14}O$ Mol. Wt. 150.22

Category : Pharmaceutical aid (preservative).

Description : Colourless crystals or white, crystal-

line powder; odour, pungent, aromatic and thyme-like; taste, pungent and aromatic.

Solubility : Very slightly soluble in *water*; freely soluble in *alcohol*, in *chloroform* and in *solvent ether*; soluble in *glacial acetic acid* and in fixed and volatile oils.

Standards : Thymol is 2-isopropyl-5-methylphenol and may be obtained from natural oils or by synthesis. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{10}H_{14}O$.

Identification : (A) A solution in *alcohol (90 per cent)* is optically inactive.

(B) When 1 g is heated in a test-tube in a water-bath with 5 ml of a 10 per cent w/v solution of *sodium hydroxide* a clear, colourless or pale-red solution is formed which becomes darker on standing, but no oily drops are separated. On adding a few drops of *chloroform* and agitating the mixture, a violet colour is produced.

(C) Dissolve a small crystal in 1 ml of *glacial acetic acid* and add 6 drops of *sulphuric acid* and 1 drop of *nitric acid*; the liquid shows a deep bluish-green colour when viewed by reflected light.

Melting range : Between 48° and 51°, Appendix 5.11.

Acidity or Alkalinity : A 4.0 per cent w/v solution in *alcohol (50 per cent)* is neutral to *litmus solution*.

Non-volatile matter : Not more than 0.05 per cent, determined on 2 g, accurately weighed, and volatilised on a water-bath and dried at 105°.

Assay : Weigh accurately about 0.1 g, transfer to an iodine flask, and dissolve in 25 ml of *N sodium hydroxide*. Add 20 ml of hot *dilute hydrochloric acid* and immediately titrate with *0.1N bromine* to within 1 to 2 ml of the calculated end-point. Warm the solution to about 75°, add two drops of *methyl orange solution* and continue the titration slowly, swirling vigorously after each addition. When the colour of the methyl orange is bleached, add two drops of *0.1N bromine*, shake well, add one drop of *methyl orange solution* and shake vigorously. If the solution is red, continue the titration, dropwise and with shaking until the red colour is discharged. Repeat the alternate addition of *0.1N bromine* and *methyl orange solution* until the red colour is discharged after the addition of the *methyl orange solution*. Each ml of *0.1N bromine* is equivalent to 0.003755 g of $C_{10}H_{14}O$.

Storage : Store in tightly-closed, light-resistant containers.

Thyroid

Thyroid Extract; Dry Thyroid

Category : Thyroid hormone.

Dose : 30 to 250 mg daily.

Description : Yellowish to buff-coloured, amorphous powder; odour, slight, meat-like and characteristic; taste, saline.

Standards : Thyroid is the cleaned, dried and powdered thyroid gland of oxen, or sheep or pigs, deprived of connective tissue and external fat. It contains not less than 0 17 per cent and not more than 0.23 per cent of iodine, I, in thyroid combination and is free from iodine in inorganic or any form of combination other than that peculiar to the thyroid gland.

Identification : When suitably mounted and examined under a microscope, Thyroid shows numerous smooth to striated hyaline fragments of colloid contents of angular to irregular shape, which are colourless to pale-yellow in water mounts, brown in *Mallory's stain* and pink in *eosin solution*, some of these fragments containing granules, minute vacuoles, crystalloidal bodies, and cells; numerous irregular fragments of follicular epithelium staining brown with *Mallory's stain*, the individual cells more or less polygonal to rounded-angular or irregularly cuboidal, often with prominent nuclei staining dark blue, their cytoplasm purplish with *delafields haematoxylin solution*. Slender, glistening segments of capillaries of closely undulate outline; numerous slender segment of neuraxons; numerous aggregrates of particles of intercellular substance and slender, mostly straight, connective tissue fibres staining blue to greenish-blue with a mixture of *Mallory's stain* and *phosphotungstic acid solution*, the bundles of fibres often appearing reddish in *Mallory's stain*; few glistening fragments of blood vessels with serrated or crenated ends as viewed in water mounts.

Inorganic iodide : To 1.0 g add 10 ml of a saturated solution of *zinc sulphate*, shake for five minutes, and filter. To 5 ml of the filtrate add 0.5 ml of *starch solution* and 0.2 ml of a 10 per cent w/v solution of *sodium nitrite*; shake, add 0.2 ml of *dilute sulphuric acid*; and shake again; no blue colour is produced.

Fat : To 1 g add 20 ml of *light petroleum (boiling range, 40° to 60°)*, shake frequently during two hours, filter, and wash the residue with two quantities each of 10 ml of *light petroleum (boiling range 40° to 60°)*, combine the extract and washings, evaporate and dry the residue to constant weight at 105°; the residue weighs not more than 30 mg.

Alcohol-soluble substances : Weigh accurately about 2 g, previously dried "in vacuo at 60°" for four hours and subsequently passed through a No. 20 *sieve* and place in a

continuous-extraction thimble containing a plug of glass wool and extract with *alcohol* for two hours. Transfer the extracted residue to a porcelain crucible, and determine the iodine contant as directed in the **Assay**, beginning at the words "add 7 g of *anhydrous potassium carbonate*.....". The residue contains not less than 95.0 per cent of the iodine content found in the **Assay**.

Microbial limits : 1 g meets the requirements of the test for the absence of *E. coli*; 10 g meet the requirements of the test for the absence of *Salmonellae*, Appendix 4.5.

Acid-insoluble ash : Not more than 0.5 per cent, Appendix 3.3.22.

Loss on drying : Not more than 7.5 per cent, determined on 0.5 g by drying "in vacuo at 60°" for four hours, Appendix 5.8.

Assay : Weigh accurately about 1 g in a porcelain crucible, add 7 g of *anhydrous potassium carbonate*, mix and overlay with 10 g more of *anhydrous potassium carbonate* and compact the mixture by tapping. Ignite for about twenty-five minutes at 650° to 700° in a pre-heated muffle furnace. Cool, add 20 ml of *water*, heat gently to boiling, and decant through a filter into a flask. Repeat the extraction by boiling with 20 ml of *water*, then wash the crucible and the char on the filter with hot *water* until the filtrate is about 200 ml in volume. Add 7 ml of freshly prepared *bromine solution*, then slowly add 40 ml of *dilute phosphoric acid* and boil until *starch iodide paper* is no longer coloured blue by the vapours. During the boiling add *water* from time to time to maintain a volume of about 200 ml. Continue boiling for five minutes, cool, add 5 ml of *phenol solution* and allow to stand for five minutes. Add 2 ml of *dilute phosphoric acid* and 5 ml of *potassium iodide solution* and titrate immediately with *0.01N sodium thiosulphate* using *starch solution* as indicator towards the end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.0002115 g of I.

Storage : Store in tightly-closed containers.

Thyroid Tablets

Category : Thyroid hormone.

Dose : Thyroid, 30 to 250 mg daily.

Usual strength : 30 mg.

Standards : Thyroid Tablets contain an amount of iodine, I, in thyroid combination equivalent to not less than 0.17 per cent and not more than 0.23 per cent of the stated amount of thyroid.

Identification : The powdered tablets exhibit the diagnostic structures described in the **Identification** test for Thyroid.

Inorganic iodide : Powder a few tablets and place a quantity of the powdered tablets equivalent to about 1 g of Thyroid in a dry test-tube. Add 10 ml of a saturated solution of *zinc sulphate*. Shake for five minutes and filter through a sintered glass filter. To 5 ml of the filtrate add 0.5 ml of *starch solution*, 4 drops of a 10 per cent w/v solution of *sodium nitrite* and 4 drops of *dilute sulphuric acid*, shaking after each addition; no blue colour is produced.

Microbial limits : The tablets meet the requirements stated under Thyroid.

Disintegration : Maximum time, 30 minutes, Appendix 5.6.1.

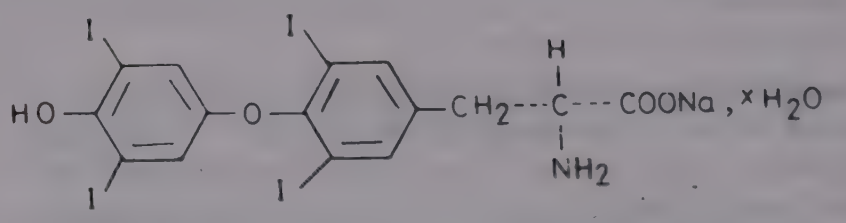
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets or more if necessary. Weigh accurately a quantity of the powder equivalent to 1 g of Thyroid in a porcelain crucible and complete the **Assay** described under Thyroid, beginning at the words "add 7 g of *anhydrous potassium carbonate*....."

Storage : Store in tightly-closed containers

Thyroxine Sodium

Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4, \times H_2O$ Mol. Wt. 798.85 (anhydrous)

Category : Thyroid hormone.

Dose : 0.05 to 0.3 mg daily.

Description : Light yellow to buff-coloured powder or fine, slightly coloured crystalline powder; odourless; tasteless.

Solubility : Very slightly soluble in *water*; soluble in solutions of alkali hydroxides and in hot solutions of alkali carbonates; slightly soluble in *alcohol*; insoluble in *chloroform*, in *acetone* and in *solvent ether*

Standards : Thyroxine Sodium is hydrated form of the sodium salt of O^4-(4-hydroxy-3,5-di-iodophenyl)-3,5-di-iodo-L-tyrosine. It contains not less than 97.0 per cent and not more than the equivalent of

103.0 per cent of $C_{15}H_{10}I_4NNaO_4$, calculated with reference to the dried substance.

Identification : (A) To 5 mg add 2 ml of equal volumes of *alcohol* and *water*, and shake well. Add one drop of *hydrochloric acid* and one drop of a 10 per cent w/v solution of *sodium nitrite* and boil; a yellow colour is produced. Cool and add *dilute ammonia solution*; the solution becomes red.

(B) *Extinction* of a 1-cm layer of a 0.01 per cent w/v solution in *0.1N sodium hydroxide* at 325 nm, about 0.76 calculated with reference to the substance dried to constant weight at 105°, Appendix 5.15 A.

(C) Ignite about 50 mg in a platinum dish over a flame ; iodine vapours are liberated; the residue gives the reactions of *sodium*, Appendix 3.1.

Clarity and colour of solution : Dissolve 1.0 g in 45 ml of a gently boiling mixture of 1 volume of *N hydrochloric acid* and 4 volumes of *ethyl alcohol*. Cool and dilute to 50.0 ml with the same solvent mixture. The solution is clear and not more intensely coloured than a mixture of 6.0 ml of *ferric chloride C.S.*, 2.5 ml of *cobalt chloride C.S.*, 1.0 ml of *copper sulphate C.S.* and sufficient *water* to produce 50.0 ml.

Specific optical rotation : Between +16° and +20°, determined at 20° in a 2.0 per cent w/v solution in a mixture of one volume of *N hydrochloric acid* and four volumes of *alcohol*, Appendix 5.12.

Soluble halides : Shake 10 mg with 10 ml of *water* containing one drop of *2N nitric acid* for five minutes and filter. Diltue the filtrate with *water* to 10 ml, and add three drops of *silver nitrate solution*; any turbidity produced is not greater than that of a control containing 0.1 ml of *0.2N hydrochloric acid*, treated in the same manner.

Loss on drying : Between 6.0 per cent and 12.0 per cent, determined on 0.5 g by drying "in vacuo at 60°" for four hours, Appendix 5.8.

Assay : Carry out the *oxygen-flask method*, Appendix 3.3.6, using about 25 mg, accurately weighed. Each ml of *0.02N sodium thiosulphate* is equivalent to 0.000666 g of $C_{15}H_{10}I_4NNaO_4$.

Storage : Store in tightly-closed, light-resistant containers.

Thyroxine Tablets

Thyroxine Sodium Tablets

Category : Thyroid hormone.

Dose : Thyroxine Sodium, 0.05 to 0.3 mg daily.

Usual strength : 0.05 mg.

Standards : Thyroxine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous thyroxine sodium, $C_{15}H_{10}I_4NNaO_4$.

Identification : Boil gently for thirty seconds a quantity of the powdered tablets equivalent to 0.5 mg of Thyroxine Sodium with a mixture of 3 ml of *alcohol (50 per cent)* and 0.2 ml of *hydrochloric acid*. Cool, filter, add 0.1 ml of a 10 per cent w/v solution of *sodium nitrite*, and boil; a yellow colour is produced. Cool and add *dilute ammonia solution*; an orange colour is produced.

Uniformity of content : Powder one tablet and transfer to a 125-ml separator with the aid of 10 ml of *5N acetic acid*. Extract vigorously for about a minute with 20 ml of a solvent mixture consisting of 30 ml of *acetic acid* and 200 ml of *isopropyl alcohol* in sufficient *chloroform* to produce 1000 ml. Transfer the lower layer to a second separator containing 30 ml of a 10 per cent w/v solution of *sodium sulphate*; add a few ml of the solvent mixture to the separator and drain it into the second separator and rinse the stem of the separator with *water*; shake the second separator vigorously for about half a minute, allow the layers to separate and transfer the lower layer to a 50-ml volumetric flask. Rinse the stem with the solvent mixture and add the rinsing to the flask. Repeat these serial extractions and transfers with a second 20-ml quantity of the solvent mixture. Rinse the stem of the second separator and dilute the combined extracts and rinsings to 50.0 ml with the solvent mixture.

Pipette an aliquot of the extract equivalent to 50 µg of Thyroxine Sodium, into a conical flask and evaporate to dryness on a water-bath in a current of air. Rinse the walls of the flask with 5 ml of *0.1N methanolic sodium hydroxide* and evaporate to dryness. Add 10 ml of *0.1N sodium hydroxide* and warm to effect solution. Chill the solution in an ice-bath. Add 2 ml of *bromine-sodium acetate solution* and sufficient *water* to produce 50 ml. Boil the solution until its volume is reduced to about 10 ml. Cool, transfer to a 25-ml volumetric flask with the aid of 10 ml of *acetate buffer, pH 3.9* and 2 ml of *water*. Add 1 ml of a 15 per cent w/v solution of *potassium iodide*, dilute to volume with *water* and mix. Measure the *extinctions* of a 1-cm layer of the resulting solution at 450 nm and at 352 nm, Appendix 5.15 A. Correct the *extinction* at 352 nm by subtracting the *extinction* at 452 nm. Multiply the result by 1.574 to convert potassium iodate to thyroxine sodium.

Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 per cent and 110 per cent of the average except that for one tablet the content may be between 85 per cent and 115 per cent of the average.

Disintegration : Maximum time, 30 minutes, Appendix 5.6.1.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.25 mg of Thyroxine Sodium, mix with 4 ml of *5N acetic acid* and warm on a water-bath. Cool, add 6 g of *chromatographic siliceous earth* and transfer quantitatively to a glass column containing a plug of glass wool. Compress the mass moderately tight. Pass through the column about 120 ml of the solvent mixture stated in the test for **Uniformity of content**. Collect the eluate in a flask and evaporate to dryness on a water-bath in a current of air. Add 5 ml of *0.1N methanolic sodium hydroxide*, rinse the walls of the flask and re-evaporate to dryness. Add 15 ml of *0.1N sodium hydroxide* and warm to effect solution. Chill the solution in an ice-bath and swirl vigorously to disperse any gelatinous precipitate. Transfer the supernatant liquid through a filter into a 50-ml volumetric flask and rinse the flask with *water* and add through the filter to the flask. Cool, dilute to volume with *water* and mix.

Pipette a quantity of the solution equivalent to 25 µg of Thyroxine Sodium into a flask, add 1 ml of *bromine-sodium acetate solution* and sufficient *water* to produce 50 ml. Complete the procedure as described in the test for **Uniformity of content**, beginning at the words "Boil the solution until its volume is reduced.....".

Storage : Store in well-closed, light-resistant containers.

Labelling : The strength is stated in terms of the equivalent amount of anhydrous thyroxine sodium.

Titanium Dioxide

TiO_2 Mol. Wt. 79.90

Category : Pharmaceutical aid and topical protectant.

Description : White or almost white infusible powder; odourless; tasteless.

Solubility : Practically insoluble in *water*, and in dilute mineral acids; slowly soluble in hot *sulphuric acid*.

Standards : Titanium Dioxide contains not less than 98.0 per cent of TiO_2, calculated with reference to the dried substance.

Identification : (A) To 0.5 g add 5 g of *anhydrous sodium sulphate* and 10 ml of *water* and mix. Add 10 ml of *sulphuric acid* and boil gently until clear; cool, add slowly 30 ml of a 25 per cent v/v solution of *sulphuric acid* and dilute with *water* to 100 ml (solution A). To 5 ml of Solution A add 0.1 ml of *strong hydrogen peroxide solution*; an orange-red colour is produced.

(B) To 5 ml of Solution A obtained in **Identification** test (A) add one piece of *granulated zinc*; after forty-five minutes a violet-blue colour is produced.

Clarity and colour of solution : Solution A obtained in **Identification** test (A) is clear to slightly opalescent and colourless.

Acidity or Alkalinity : Shake 5.0 g with 50 ml of *carbon dioxide-free water* for five minutes, and centrifuge or filter until a clear solution is obtained; 10 ml of the solution requires for neutralization not more than 1.0 ml of *0.01N hydrochloric acid* or not more than 1.0 ml of *0.01N sodium hydroxide* using 0.1 ml of *bromothymol blue solution* as indicator.

Water-soluble matter : Not more than 0.5 per cent, determined by the following method: Boil 10.0 g for five minutes with 150 ml of *water* containing 0.5 g of *ammonium sulphate*. Cool, dilute to 200 ml with *water* and filter until a clear solution is obtained. Evaporate 100 ml of the filtrate to dryness and ignite.

Arsenic : Not more than 8 parts per million, Appendix 3.2.1.

Barium : Not more than 5 parts per million, determined by the following method: Shake 20 g for one minute with 30 ml of *hydrochloric acid*, add 100 ml of *water* and boil. Filter while hot through a hardened filter until a clear filtrate is obtained. Wash the filter with 60 ml of *water* and dilute the combined filtrate and washings to 200 ml with *water*. To 10 ml of the resulting solution add 1 ml of *dilute sulphuric acid*; the solution remains clear for not less than thirty minutes.

Heavy metals : Not more than 20 parts per million, determined by the following method: Dilute 10 ml of the solution prepared in the test for **Barium** to 20 ml with *water*. To 12.0 ml of the solution add 1.2 ml of *thioacetamide reagent* and 2 ml of *acetate buffer, pH 3.5*, mix, and allow to stand for two minutes. Any brown colour produced is not more intense than that produced by similarly treating a mixture of 1.0 ml of *standard lead solution* and 2.0 ml of the solution being examined.

Iron : Not more than 200 parts per million, determined by the following method: To 8 ml of Solution A obtained in **Identification** test (A) add 4 ml of *water* and one drop of *bromine water*. After five minutes remove the excess of bromine by passing a current of air and add 3 ml of *potassium thiocyanate solution* and shake. After five minutes, any red colour in the solution is not more intense than the colour obtained by treating 0.4 ml of *standard iron solution* and 8 ml of a 20 per cent w/v solution of *sulphuric acid* in the same manner.

Loss on drying : Not more than 0.5 per cent, determined on 2.0 g by drying in an oven at 105° for three hours, Appendix 5.8.

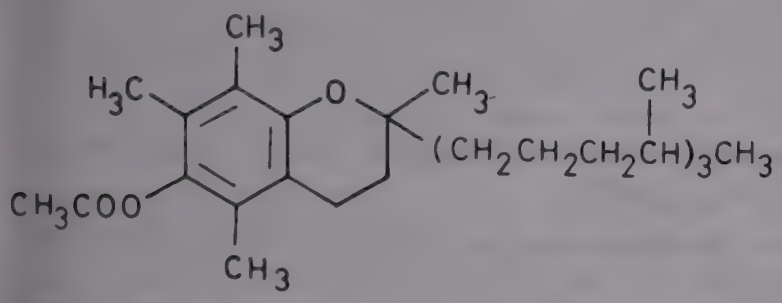
Loss on ignition : Weigh accurately about 2.0 g previously dried at 105° for three hours and ignite at 800 ± 25° to constant weight; it loses not more than 0.5 per cent of its weight.

Assay : Weigh accurately about 0.3 g, transfer to a beaker, and add 20 ml of *sulphuric acid* and 8 g of *ammonium sulphate*. Mix and heat until white fumes appear; continue heating over a strong flame until solution is effected. Cool and carefully dilute with 100 ml of *water*. Heat gently and bring to boil with continuous stirring. Cool, filter and wash with several quantities each of 10 ml of *water*. Combine the filtrate and washings, add 10 ml of *strong ammonia solution*, cool and dilute to 200.0 ml with *water*. Pipette 50 ml of the resulting solution into a flask, add 100 ml of *water* and 4 ml of *strong hydrogen peroxide solution*. Cool and add 50.0 ml of *0.05M disodium ethylenediamine tetraacetate*. Allow to stand for five minutes, adjust the pH of the solution to 5.0 with *sodium hydroxide solution*, add 5 g of *hexamine* and titrate with *0.05M zinc chloride* using *xylenol orange solution* as indicator. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.003995 g of TiO_2.

Storage : Store in well-closed containers. Avoid contact with aluminium.

Tocopheryl Acetate

α-Tocopheryl Acetate; Vitamin E Acetate



$C_{31}H_{52}O_3$ Mol. Wt. 472.75

Category : Vitamin E Supplement.

Dose : Prophylactic, from 5 to 30 mg.

Therapeutic, to be determined according to the needs of the patient.

Description : Clear, slightly greenish-yellow, viscous, oily liquid; almost odourless.

Solubility : Practically insoluble in *water*; soluble in *acetone*, in *chloroform*, in *alcohol*, in *solvent ether* and in fatty oils.

Standards : Tocopheryl Acetate is the dl-form of (±)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanyl acetate. It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of $C_{31}H_{52}O_3$.

Identification : (A) Dissolve 0.2 g in 2 ml of *ethyl alcohol*. Add 0.2 ml of *nitric acid* and heat carefully on a water-bath for five minutes. The colour changes from yellow to brick-red.

(B) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.01 per cent w/v solution in *ethyl alcohol* exhibits a maximum at about 284 nm; *extinction* at 284 nm, about 0.43, Appendix 5.15 A.

Refractive index : Between 1.494 and 1.498, determined at 20°, Appendix 5.14.

Acid value : Not more than 2.0, determined on 2.0 g, Appendix 3.3.15.

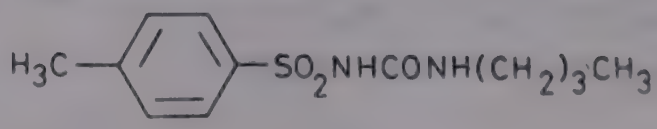
Free tocopherol : Not more than 2.0 per cent, determined by the following method: Weigh accurately about 0.5 g and dissolve in 50 ml of *alcohol*. Titrate with *0.01N ceric ammonium sulphate* using 0.1 ml of *diphenylamine solution* as indicator, until a blue colour persisting for at least ten seconds is obtained. Carry out a blank determination and make any necessary correction. Each ml of *0.01N ceric ammonium sulphate* is equivalent to 0.002154 g of tocopherol.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Assay : Weigh accurately about 0.3 g and dissolve in 25 ml of *alcohol*. Add 20 ml of *5N alcoholic sulphuric acid* and heat on a water-bath under a reflux condenser for three hours. Cool, transfer the solution quantitatively to a 200-ml graduated flask. Rinse the apparatus with *alcohol* and add the rinsings to the flask. Make upto volume with *alcohol*. Pipette 25.0 ml of the resulting solution into a 100-ml flask, add 25 ml of *0.5N alcoholic sulphuric acid* and 10 ml of *water*. Titrate with *0.01N ceric ammonium sulphate*, using 0.1 ml of *diphenylamine solution* as indicator, until a blue colour persisting for at least 10 seconds is obtained. Carry out a blank determination, omitting the substance being tested and make any necessary correction. Each ml of *0.01N ceric ammonium sulphate* is equivalent to 0.002364 g of $C_{31}H_{52}O_3$.

Storage : Store in well-closed, light-resistant containers.

Tolbutamide



$C_{12}H_{18}N_2O_3S$ Mol. Wt. 270.35

Category : Antidiabetic.

Dose : 0.5 to 1.5 g, daily in divided doses.

Description : White or practically white, crystalline powder; almost odourless; taste, slightly bitter.

Solubility : Practically insoluble in *water*; soluble in *alcohol* and in *chloroform*.

Standards : Tolbutamide is 1-butyl-3-tosylurea. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{12}H_{18}N_2O_3S$, calculated with reference to the dried substance.

Identification : (A) Boil 0.1 g with 8 ml of 50 per cent v/v solution of *sulphuric acid* under a reflux condenser for thirty minutes. Make the solution strongly alkaline with *sodium hydroxide solution* and steam distil for thirty minutes, receiving the distillate in 20 ml of *0.1N hydrochloric acid*. To 1 ml of the solution containing the distillate add 0.1 g of *sodium acetate* and 10 ml of *buffer solution, pH 9.4*. Cool in an ice-bath for ten minutes, add 1 ml of *diazotised nitroaniline solution*; set aside for twenty minutes and add dropwise 1 ml of *sodium hydroxide solution*; an orange-red colour is produced.

(B) Boil 0.1 g with 8 ml of a 50 per cent v/v solution of *sulphuric acid* under a reflux condenser for thirty minutes. Cool in an ice-bath; a crystalline precipitate of *p*-toluenesulphonylamide is formed, which after recrystallisation from hot *water* and drying at 105° has a melting range between 135° and 138°, Appendix 5.11.

(C) The light absorption, in the range 230 to 350 nm of a 1-cm layer of a 0.001 per cent w/v solution in *methyl alcohol* exhibits a maximum only at 228 nm; *extinction* at 228 nm, about 0.49, Appendix 5.15 A.

Melting range : Between 126° and 132°, Appendix 5.11.

Non-sulphonyl urea : Dissolve 0.5 g in 1 ml of *dilute ammonia solution* and 9 ml of *water*; not more than a faint opalescence occurs.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for three hours, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and dissolve in a mixture of 40 ml of *alcohol* and 20 ml of *water*. Titrate with *0.1N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.02704 g of $C_{12}H_{18}N_2O_3S$.

Storage : Store in well-closed containers.

Tolbutamide Tablets

Category : Antidiabetic.

Dose : Tolbutamide, 0.5 to 1.5 g daily.

Usual strength : 0.5 g.

Standards : Tolbutamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Tolbutamide, $C_{12}H_{18}N_2O_3S$.

Identification : (A) Extract a quantity of the powdered tablets equivalent to 1 g of Tolbutamide with five quantities, each of 4 ml, of *chloroform*, filter and evaporate to dryness on a water-bath; the residue complies with **Identification** test (B) described under Tolbutamide.

(B) Boil 0.2 g of the residue obtained in **Identification** test (A), with 8 ml of *sulphuric acid (50 per cent w/w)* under a reflux condenser for thirty minutes, cool and filter. Make the filtrate alkaline with *sodium hydroxide solution* and heat; the odour of butylamine is noticed.

Disintegration time : Maximum time, 30 minutes, Appendix 5.6.1.

Dissolution : Comply with the *dissolution test for tablets and capsules*, Appendix 5.7, using as the medium 1000 ml of a solution containing 2.04 per cent w/v of *disodium hydrogen phosphate* and 0.135 per cent w/v of *potassium dihydrogen phosphate*, and placing one tablet in the basket for each test. Withdraw a sample of 10 ml of the medium and filter. Measure the *extinction* of the filtered solution, suitably diluted if necessary, at the maximum at about 228 nm, Appendix 5.15 A. Calculate the content of $C_{12}H_{18}N_2O_3S$, in the medium, taking 417 as the value of E(1 per cent, 1-cm) at the maximum at about 228 nm.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 0.5 g of Tolbutamide, add 50 ml of *alcohol* previously neutralised to *phenolphthalein solution*, warm to dissolve, add 25 ml of *water*, and titrate with *0.1N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.02704 g of $C_{12}H_{18}N_2O_3S$.

Storage : Store in well-closed containers.

Tragacanth

Gum Tragacanth

Category : Pharmaceutical aid (suspending agent).

Description : Thin, flattened, ribbon-like strips, frequently curved or straight or spirally twisted

linear pieces or fragments, white to pale yellow in colour, translucent, horny and with short fracture; odourless; taste, insipid and mucilaginous.

Examined under a microscope, the powdered material shows in the gummy mass numerous stratified cellular membranes and a few starch grains.

Standards : Tragacanth is the air-hardened gummy exudation from *Astragalus gummifer* Labillardiere, or other species of *Astragalus* (Fam. Leguminosae).

Identification : (A) Add 1 g to 50 ml of *water*; it swells and forms a smooth, nearly uniform, stiff, opalescent mucilage free from cellular fragments.

(B) Moisten 0.5 g of the powdered material with 1 ml of *alcohol* and add gradually, while shaking, 50 ml of *water* until a homogenous mucilage is obtained. To 5 ml of the mucilage add 5 ml of *water* and 2 ml of *barium hydroxide solution*. A slightly flocculent precipitate is formed which, when heated for ten minutes on a water-bath, gives an intense yellow colour.

(C) Add 4 ml of a 0.5 per cent w/v dispersion in *water* to 0.5 ml of *hydrochloric acid* and heat on a water-bath for thirty minutes. To one half of the resulting liquid, add 1.5 ml of *sodium hydroxide solution* and 3 ml of *alkaline cupric tartrate solution* and heat on a water-bath. A reddish-brown precipitate is formed. To the other half of the liquid, add a few drops of *barium chloride solution*. No precipitate is formed (freedom from agar).

Acacia and other soluble gums : To 20 ml of a 2.5 per cent w/v suspension of the powdered material prepared with freshly boiled *water*, add 10 ml of *lead acetate solution*. A flocculent precipitate is formed. Filter and add to the filtrate 10 ml of *lead subacetate solution*. A slight cloudiness may appear, but there is no precipitate.

Karaya gum : Boil 1 g with 20 ml of *water* until a mucilage is formed, add 5 ml of *hydrochloric acid*, and again boil for five minutes; no pink or red colour develops.

Sterculia gum : (a) Shake 0.2 g of the powdered material with 10 ml of *alcohol (60 per cent)* in a 10-ml cylinder. Any gel formed occupies not more than 1.5 ml.

(b) Shake 1.0 g of the powdered material with 100 ml of *water* and titrate with *0.01 N sodium hydroxide*, using *methyl red solution* as indicater. Not more than 5.0 ml is required.

Foreign matter : Not more than 1.0 per cent, determined by the following method: Weigh accurately about 5 g and disperse in 65 ml of *dilute hydrochloric acid* and 35 ml of *water*. Heat cautiously until the solution can be filtered on adding enough *water* to maintain the original volume. Filter through a sintered-glass filter and wash the filter with *water* until the washings no longer give turbidity with *silver nitrate solution*. Dry at 100° to 105°, cool and weigh.

Arsenic : Not more than 3 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 40 parts per million, determined on 0.5 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 4.0 per cent, Appendix 3.2.7.

Storage : Store in well-closed containers.

POWDERED TRAGACANTH

Category : Pharmaceutical aid (suspending agent).

Description : White to yellowish-white angular fragments with few or no lignited vegetable tissues. When examined in water mounts it shows angular fragments of mucilage with circular or irregular lamellae and occasional starch grains.

Standards; Identification; Acacia and other soluble gums; Karaya gum; Sterculia gum; Foreign matter; Arsenic; Heavy metals: Complies with the requirements stated under Tragacanth.

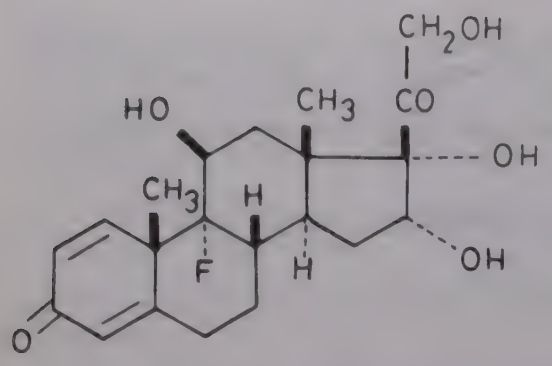
Apparent viscosity : Weigh accurately 0.88 g, transfer to a dry 500-ml conical flask and add 5 ml of *alcohol*. Ensure that the gum is completely wetted and evenly dispersed over the inner surface of the flask, add 195 ml of *water* as quickly as possible, and shake. Allow to stand for one hour, swirling frequently. Attach a reflux condenser to the flask and immerse in vigorously boiling water so that the surface of the water is about 2.5 cm above the surface of the mucilage. Continue heating for one hour, gently swirling at intervals of fifteen minutes without removing the flask from the water. Remove the flask from the water, stopper, shake frequently during a period of two hours and allow to stand for twenty-four hours. Determine the efflux time, at 20°, of 50 ml using a Redwood No. 1 viscometer. Multiply the mean of six readings by $27/x$, where x is the efflux time, at 20°, of 50 ml of *water* determined in the same instrument. The corrected efflux time is not less than 100 seconds.

Microbial limits : 1.0 g if free from *E. coli*, and *Salmonellae*, Appendix 4.5.

Sulphated ash : Not more than 5.0 per cent, Appendix 3.2.7.

Storage : Store in well-closed containers.

Triamcinolone



$C_{21}H_{27}FO_6$ Mol. Wt. 394.44

Category : Adrenocortical steroid (anti-inflammatory).

Dose : 4 to 12 mg, one to four times daily.

Description : White or practically white, crystalline powder; odourless.

Solubility : Very slightly soluble in *water*, in *chloroform*, and in *solvent ether*; slightly soluble in *alcohol*, and in *methyl alcohol*.

Standards : Triamcinolone is 9*α*-fluoro-11*β*,16*α*-17*α*, 21-tetrahydroxypregna-1,4-diene-3,20-dione. It contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of $C_{21}H_{27}FO_6$, calculated with reference to the dried substance.

Identification : (A) Dissolve 1 mg in 6 ml of *alcohol*, add 5 ml of a 1 per cent w/v solution of *di-t-butyl-p-cresol* in *alcohol* and 5 ml of *dilute sodium hydroxide solution* and heat on a water-bath under a reflux condenser for 20 minutes; a pinkish-lavender colour is produced.

(B) Complies with the test for *identification of steroids* using *solvent I* and *mobile phase A*, Appendix 3.3.11.

Specific optical rotation : Between +65° and +71°, determined at 20°, in a 1.0 per cent w/v solution in *dimethylformamide*, Appendix 5.12.

Related foreign steroids : Complies with the test for *related foreign steroids, Method A*, Appendix 3.3.12.

Heavy metals : Not more than 25 parts per million, determined by Method B on 0.8 g, Appendix 3.2.4.

Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.2 per cent, determined on 0.5 g by drying "in vacuo at 60°" for three hours, Appendix 5.8.

Assay : Weigh accurately about 50 mg and dissolve in sufficient *alcohol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 50.0 ml with *alcohol* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 238 nm, Appendix 5.15 A. Calculate the content of $C_{21}H_{27}FO_6$, taking 380 as the value of E(1 per cent, 1-cm) at the maximum at about 238 nm.

Storage : Store in well-closed containers.

Triamcinolone Tablets

Category : Adrenocortical steroid.

Dose : Triamcinolone, 4 to 12 mg, one to four times daily.

Usual strengths : 2 mg; 4 mg; 8 mg.

Standards : Triamcinolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Triamcinolone, $C_{21}H_{27}FO_6$.

Identification : Extract a quantity of the powdered tablets equivalent to about 2 mg of Triamcinolone, with 50 ml of *alcohol*, filter and evaporate 20 ml of the filtrate to dryness. The residue complies with **Identification** test (A) described under Triamcinolone.

Related foreign steroids : On a quantity of the powdered tablets equivalent to about 4 mg of Triamcinolone carry out the test for **Related foreign steroids**, described under Betamethasone Tablets.

Uniformity of content : Powder one tablet, add 50 ml of *ethyl alcohol*, shake for thirty minutes and add sufficient *ethyl alcohol* to produce 100.0 ml. Centrifuge and pipette a suitable volume of the supernatant liquid equivalent to 0.5 mg of Triamcinolone and dilute to 50.0 ml with *ethyl alcohol*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 238 nm, Appendix 5.15 A. Calculate the content of $C_{21}H_{27}FO_6$, taking 380 as the value of E(1 per cent, 1-cm) at the maximum at about 238 nm.

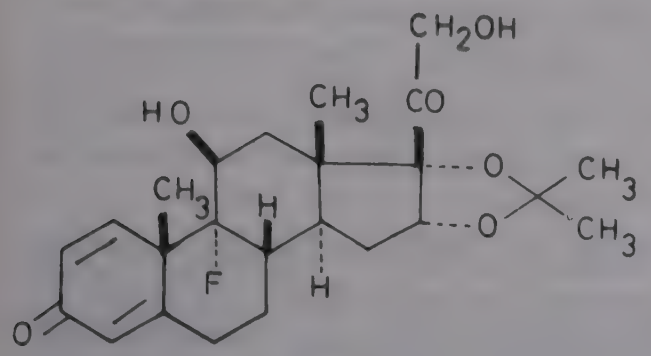
Repeat the operation with a further nine tablets and calculate the average content of the ten tablets. The content of each tablet is between 90 per cent and 110 per cent of the average except that for one tablet the content may be between 85 per cent and 115 per cent of the average.

Other requirements : Comply with the requirements stated under Tablets.

Assay : Carry out the **Assay** described under Prednisolone Tablets, using *triamcinolone R.S.* for preparing the *standard solution*.

Storage : Store in well-closed containers.

Triamcinolone Acetonide



$C_{24}H_{31}FO_6$ Mol. Wt. 434.50

Category : Adrenocortical steroid (topical anti-inflammatory).

Description : White or cream-coloured crystalline powder; odourless or almost odourless.

Solubility : Very slightly soluble in *water*; slightly soluble in *alcohol*; sparingly soluble in *chloroform*; soluble in *acetone*.

Standards : Triamcinolone Acetonide is 9α-fluoro-11 β, 21-dihydroxy-16α, 17α-isopropylidenedioxy-pregna-1,4-diene-3,20-dione. It contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of $C_{24}H_{31}FO_6$, calculated with reference to the dried substance.

Identification : (A) Complies with the test for *identification of steroids*, Appendix 3.3.11, using *solvent I* and *mobile phase B*.

(B) Complies with **Identification** test (C) described under Betamethasone.

Specific optical rotation : Between +101° and +107°, determined at 20°, in a 1.0 per cent w/v solution in *dioxan*, Appendix 5.12.

Related foreign steroids : Complies with the test for *related foreign steroids, Method A*, Appendix 3.3.12.

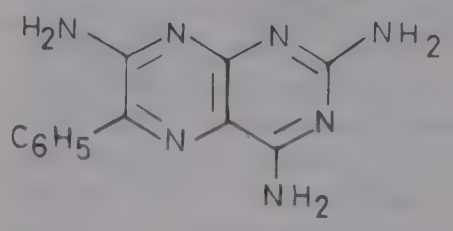
Sulphated ash : Not more than 0.2 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.5 per cent, determined on 0.5 g by drying "in vacuo at 60°", Appendix 5.8.

Assay : Weigh accurately about 25 mg and dissolve in sufficient *ethyl alcohol* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *ethyl alcohol* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 240 nm, Appendix 5.15 A. Calculate the content of $C_{24}H_{31}FO_6$, taking 354 as the value of E(1 per cent, 1-cm) at the maximum at about 240 nm.

Storage : Store in well-closed, light-resistant containers.

Triamterene



$C_{12}H_{11}N_7$ Mol. Wt. 253.27

Category : Diuretic.

Dose : 150 to 250 mg daily, in divided doses.

Description : Yellow, crystalline powder; odourless; almost tasteless at first, becoming slightly bitter.

Solubility : Very slightly soluble in *water*; in *alcohol* and in *chloroform*; insoluble in *solvent ether*.

Standards : Triamterene is 2,4,7-triamino-6-phenylpteridine. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{12}H_{11}N_7$, calculated with reference to the dried substance.

Identification : (A) The light absorption, in the range 230 to 365 nm, of a 1-cm layer of a 0.0005 per cent w/v solution containing 10 per cent v/v of *acetic acid*, exhibits a maximum only at 360 nm; *extinction* at 360 nm, about 0.42, 5.15 A.

(B) An acidified solution has an intense bluish fluorescence.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 8 volumes of *ethyl acetate*, 1 volume of *methyl alcohol* and 1 volume of *glacial acetic acid* as the mobile phase. Apply separately to the plate immediately after it has been heated and allowed to cool, 5 µl of each of the following solutions: For solution (1) dissolve 0.1 g of the substance being examined in sufficient *dimethyl sulphoxide* to produce 20.0 ml and dilute 2.0 ml to 50.0 ml with *methyl alcohol*. For solution (2) dissolve 5 mg of *2,7-diamino-4-hydroxy-6-phenylpteridine R.S.* and 5 mg of *2,4-diamino-7-hydroxy-6-phenylpteridine R.S.* in sufficient *dimethyl sulphoxide* to produce 100 ml and dilute 2.0 ml to 100 ml with *methyl alcohol*.

After removal of the plate, allow it to dry until the odour of the solvent is no longer detectable and examine under an ultra-violet lamp having a maximum output at about 366 nm. The spots in the chromatogram obtained with solution (2) are more intense than any corresponding spots in the chromatogram obtained with solution (1).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.15 g and dissolve in 100 ml of *glacial acetic acid*. Titrate with *0.1N perchloric acid* determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02533 g of $C_{12}H_{11}N_7$.

Storage : Store in well-closed containers.

Triamterene Capsules

Category : Diuretic.

Dose : Triamterene, 150 to 250 mg daily, in divided doses.

Usual strength : 50 mg.

Standards : Triamterene Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Triamterene, $C_{12}H_{11}N_7$.

Identification : The solution obtained in the **Assay** has a bluish fluorescence and the light absorption in the range 230 to 365 nm, exhibits a maximum only at 360 nm, Appendix 5.15 A.

Related substances : Complies with the test described under Triamterene, using as solution (1), a solution prepared in the following manner : Dissolve a quantity of the contents of the capsules equivalent to 0.1 g of Triamterene in sufficient *dimethyl sulphoxide* to produce 20.0 ml and dilute 2.0 ml to 50.0 ml with *methyl alcohol*.

Other requirements : Comply with the requirements stated under Capsules.

Assay : Weigh accurately a quantity of the mixed contents of 20 capsules equivalent to 0.1 g of Triamterene and dissolve in 50 ml of a mixture of equal parts of *glacial acetic acid* and *water* with the aid of gentle heat, cool and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with *dilute acetic acid* and measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 360 nm, Appendix 5.15 A. Calculate the content of $C_{12}H_{11}N_7$, from the *extinction* obtained from a suitable aliquot of a solution of *triamterene R.S.* in *dilute acetic acid* treated in a similar manner.

Storage : Store in well-closed containers.

Trichloroethylene

$CHCl:CCl_2$ Mol. Wt. 131.40

Category : Analgesic; light anaesthetic.

Description : Clear, colourless or blue, mobile liquid; odour, characteristic and resembling that of chloroform; taste, sweet and burning. Slowly decomposed by light in the presence of moisture.

Solubility : Practically insoluble in *water*; miscible with *alcohol*, with *chloroform* and with *solvent ether*.

Standards : Trichloroethylene contains not less than 99.5 per cent and not more than 100.0 per cent of C_2HCl_3. It contains not less than 0.008 per cent and not more than 0.012 per cent w/w of Thymol, as a preservative. It may also contain not more than 0.001 per cent w/w of a blue colour permitted under the Drugs and Cosmetic Rules, 1945.

Identification : (A) Boiling range, between 86° and 88°, Appendix 5.3.

(B) Transfer 5 ml to a glass-stoppered cylinder, add 5 ml of *bromine water* and shake the mixture vigorously at intervals of fifteen minutes; at the end of one hour a white turbid solution forms in the lower layer (chloroform and carbon tetrachloride remain clear).

Specific gravity : Between 1.458 and 1.463, Appendix 5.19, indicating between 99.5 per cent and 100.0 per cent of C_2HCl_3.

Acidity or Alkalinity : To 100 ml of freshly boiled and cooled *water* in a glass-stoppered flask add a few drops of *bromocresol purple solution* and, if necessary, neutralise by dropwise addition of *0.1N sodium hydroxide* or *0.1N hydrochloric acid*. Transfer 50 ml of the neutralised water to another flask, add 100 ml of the substance to be examined and shake. The colour of the aqueous layer is identical with that of the neutralised water. If more alkaline, titrate the mixed liquids by dropwise addition of *0.1N hydrochloric acid*, shaking after each addition, until the colour of the aqueous layer matches that of the neutralised water. Not more than 1.0 ml of *0.1N hydrochloric acid* is required.

Chlorides : Shake 10 ml with 20 ml of freshly boiled and cooled *water* for three minutes. To 5 ml of the aqueous layer add 5 ml of *water*, one drop of *nitric acid* and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free chlorine, phosgene, acetylenic compounds : Complies with the requirements stated under Tetrachloroethylene.

Non-volatile matter : Not more than 0.003 per cent w/v, determined by evaporating 50.0 ml on a water-bath, and drying the residue at 105° for two hours.

Thymol : Weigh accurately about 3 ml in a 100 ml volumetric flask containing 5 ml of a 1.0 per cent w/v solution of *sodium hydroxide* and mix by gentle swirling. Evaporate the Trichloroethylene in a stream of nitrogen, and add 10 ml of *buffer solution, pH 8.0* and 1.0 ml of *chlorimide solution*. Swirl gently, allow to stand for

exactly fifteen minutes, then add 3 ml of a 1.0 per cent w/v solution of *sodium hydroxide* and dilute to volume with *water*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 590 nm against a blank prepared in a similar manner omitting the substance to be examined, Appendix 5.15 A. Calculate the content of thymol, from the *extinction* obtained by carrying out the determination simultaneously on a solution of *thymol R.S.* in a 1.0 per cent w/v solution of *sodium hydroxide*.

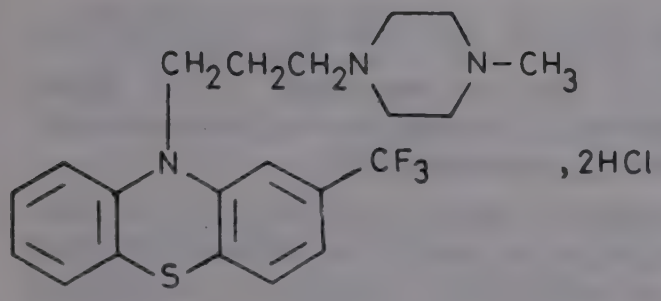
Storage : Store in tightly-closed, light-resistant containers in a cool place. Dispense in the original container.

Labelling : The label on the container states in a prominent place.

CAUTION—Repeated use may cause liver damage.

WARNING—Not to be used in any closed-circuit rebreathing system utilising soda lime or other alkali because phosgene or other toxic products may be formed.

Trifluoperazine Hydrochloride



$C_{21}H_{24}F_3N_3S$, 2HCl Mol. Wt. 480.42

Category : Antipsychotic agent.

Dose : Oral, the equivalent of 2 to 30 mg of trifluoperazine daily, in divided doses; by intramuscular injection, the equivalent of 1 to 2 mg of trifluoperazine every 4 to 6 hours as required.

Description : White to pale yellow, crystalline powder; odourless or almost odourless; taste, bitter.

Solubility : Freely soluble in *water*; soluble in *alcohol*; sparingly soluble in *chloroform*; insoluble in *solvent ether*, and in *benzene*.

Standards : Trifluoperazine Hydrochloride is the dihydrochloride of 10-[3-(4-methylpiperazin-1-yl)-propyl]-2-trifluoromethylphenothiazine. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{21}H_{24}F_3N_3S$, 2HCl, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *trifluoperazine hydrochloride R.S.*, Appendix 5.15 B.

(B) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.0008 per cent w/v solution in *0.01N hydrochloric acid* exhibits a maximum at 256 nm and a less well-defined maximum at about 305 nm; *extinction* at 256 nm, about 0.5, Appendix 5.15 A.

(C) Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for five minutes; an orange colour is produced.

(D) Dissolve 20 mg in 2 ml of *water* and add one drop of *ferric chloride test-solution*; an amber colour is produced.

(E) A solution (1 in 10) gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 1.7 and 2.6, determined in a 5.0 per cent w/v solution, Appendix 5.10.

Foreign substances : Complies with the test described under Chlorpromazine Hydrochloride.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

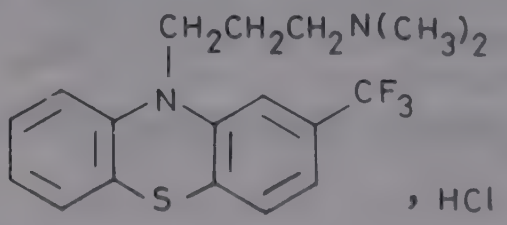
Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying "in vacuo at 60°", Appendix 5.8.

Assay : Weigh accurately about 0.4 g and dissolve in 50 ml of *glacial acetic acid*, add 10 ml of *mercuric acetate solution*, two drops of *crystal-violet solution* and titrate with *0.1N perchloric acid* to a blue-green end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.02402 g of $C_{21}H_{24}F_3N_3S$, 2HCl.

Storage : Store in tightly-closed, light-resistant containers.

Trifluprazine Hydrochloride

Flupromazine Hydrochloride



$C_{18}H_{19}F_3N_2S$, HCl Mol. Wt. 388.88

Category : Antipsychotic agent.

Dose : Oral, the equivalent of 30 to 150 mg of trifluprazine daily.

By intramuscular injection, the equivalent of 5 to 10 mg of triflupromazine repeated every four hour, if necessary.

By intravenous injection, the equivalent of 1 to 3 mg of triflupromazine repeated every four hours, if necessary.

Description : White to pale yellowish-brown, crystalline powder; odour, slight and characteristic.

Solubility : Soluble in *water*, in *alcohol*, and in *acetone*; insoluble in *solvent ether*.

Standards : Triflupromazine Hydrochloride is 10-(3-dimethylaminopropyl) 2-trifluoromethylphenothiazine monohydrochloride. It contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of $C_{18}H_{19}F_3N_2S, HCl$, calculated with reference to the dried substance.

Identification : (A) The *infra-red absorption spectrum* exhibits maxima which are only at the same wavelengths as, and have similar relative intensities to, those in the spectrum of *triflupromazine hydrochloride R.S.*, Appendix 5.15 B.

(B) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *0.1N hydrochloric acid* exhibits a maximum at 255 nm and a less well-defined maximum at about 305 nm; *extinction* at 255 nm, about 0.63, Appendix 5.15 A.

(C) A solution (1 in 10) gives the reactions of *chlorides*, Appendix 3.1.

Melting range : Between 170° and 178°, Appendix 5.11.

Foreign substances : Complies with the test described under Chlorpromazine Hydrochloride.

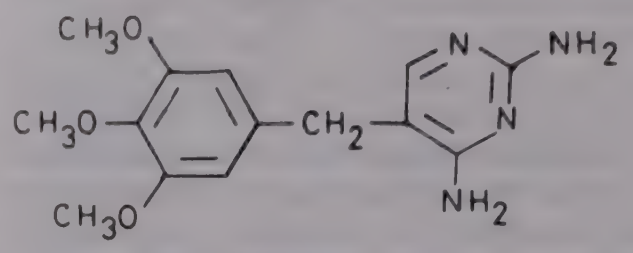
Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° for two hours, Appendix 5.8.

Assay : Weigh accurately about 0.8 g and dissolve in 50 ml of *glacial acetic acid*; add 10 ml of *mercuric acetate solution*, two drops of *crystal-violet solution* and titrate with *0.1N perchloric acid* to a blue-green end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.03889 of $C_{18}H_{19}F_3N_2S, HCl$.

Storage : Store in well-closed, light-resistant containers.

Trimethoprim



$C_{14}H_{18}N_4O_3$ Mol. Wt. 290.32

Category : Antibacterial.

Description : White to yellowish-white powder; odourless; taste, very bitter.

Solubility : Very slightly soluble in *water*; slightly soluble in *alcohol*, in *chloroform* and in *methanol*; practically insoluble in *solvent ether*.

Standards : Trimethoprim is 5-(3,4,5-trimethoxy benzyl)-pyrimidine-2,4-diamine. It contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of $C_{14}H_{18}N_4O_3$, calculated with reference to the dried substance.

Identification : The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.002 per cent w/v solution in *0.1N sodium hydroxide solution* exhibits a maximum only at 287 nm; *extinction* at 287 nm, about 0.49, Appendix 5.15 A.

Melting range : Between 199° and 203°, Appendix 5.11.

pH : Between 7.5 and 8.5, determined in the filtrate obtained by shaking 0.2 g with 20 ml of *water* for one minute and filtering, Appendix 5.10.

Related substances : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 90 volumes of *chloroform*, 8 volumes of *methyl alcohol*, and 2 volumes of *strong ammonia solution* as the mobile phase. Apply separately to the plate (1) 50 µl of a 1.0 per cent w/v solution of the substance being examined in *chloroform* and (2) 2 µl of a 0.05 per cent w/v solution of the substance being examined in *chloroform*. After removal of the plate, allow it to dry in air, and examine under ultra-violet lamp having a maximum output at about 254 nm. Any spot in the chromatogram obtained with solution (1), other than the principal spot, is not more intense than the spot in the chromatogram obtained with solution (2).

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying "in vacuo at 100°", Appendix 5.8.

Assay : Weigh accurately about 0.4 g and dissolve in 50 ml of *glacial acetic acid* with the aid of gentle heat. Cool and titrate with *0.1N perchloric acid* and determine the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of

0.1N perchloric acid is equivalent to 0.02903 g of $C_{14}H_{18}N_4O_3$.

Storage : Store in well-closed containers.

Trimethoprim and Sulphamethoxazole Tablets

Cotrimoxazole Tablets

Category : Antibacterial.

Dose : Trimethoprim, 160 to 480 mg and Sulphamethoxazole 800 mg to 2.4 g daily, in divided doses.

Usual strengths : Tablets containing in each 20 mg of Trimethoprim and 100 mg of Sulphamethoxazole, 80 mg of Trimethoprim and 400 mg of Sulphamethoxazole, and 160 mg of Trimethoprim and 800 mg of Sulphamethoxazole.

Standards : Trimethoprim and Sulphamethoxazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amounts of Trimethoprim, $C_{14}H_{18}N_4O_3$ and Sulphamethoxazole, $C_{10}H_{11}N_3O_3S$. The tablets may be coated.

Identification : Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 20 volumes of *chloroform*, 2 volumes of *methyl alcohol* and one volume of *dimethylformamide* as the mobile phase. Apply separately to the plate 5 µl each of the following three solutions: For solution (1) shake a quantity of the powdered tablets equivalent to 0.4 g of Sulphamethoxazole with 20 ml of *methyl alcohol* and filter; solution (2) is a 2.0 per cent w/v solution of *sulphamethoxazole R.S.* in *methyl alcohol*; solution (3) is a 0.4 per cent w/v solution of *trimethoprim R.S.* in *methyl alcohol*. After removal of the plate, allow it to dry in air, and spray with *dilute potassium iodobismuthate solution*.

One of the principal spots in the chromatogram obtained with solution (1) corresponds to the spot in the chromatogram obtained with solution (2) and the other corresponds to the spot in the chromatogram obtained with solution (3).

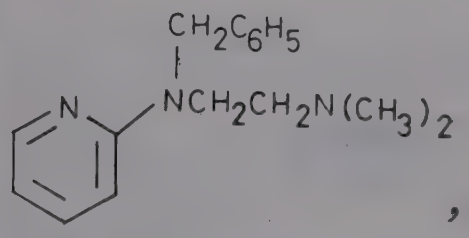
Other requirements : Comply with the requirements stated under Tablets.

Assay : (1) *For Trimethoprim* – Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 0.05 g of Trimethoprim, add 30 ml of *0.1N sodium hydroxide*, mix, extract with four successive quantities, each of 50 ml, of *chloroform*, washing each extract with same two quantities, each of 10 ml, of *0.1N sodium hydroxide*. Extract the chloroform solution with four successive quantities, each of 50 ml, of *dilute acetic acid*, wash the combined aqueous extracts with 5 ml of *chloroform*, and dilute the aqueous solution to 250.0 ml with *dilute acetic acid*; to 10.0 ml of this solution add 10 ml of *dilute acetic acid*, dilute to 100.0 ml with *water*, and measure the *extinction* of a 1-cm layer at the maximum at about 271 nm, Appendix 5.15 A, using as the blank a solution prepared by diluting 20.0 ml *dilute acetic acid* to 100.0 ml with *water*. Calculate the content of $C_{14}H_{18}N_4O_3$, taking 204 as the value of E(1 per cent, 1-cm) at the maximum at about 271 nm.

(2) *For Sulphamethoxazole* – Weigh and powder 20 tablets. Dissolve a quantity of the powder equivalent to 0.5 g of Sulphamethoxazole in 60 ml of *water* and 10 ml of *hydrochloric acid* and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1M sodium nitrite* is equivalent to 0.02533 g of $C_{10}H_{11}N_3O_3S$.

Storage : Store in well-closed containers.

Tripelennamine Hydrochloride



$C_{16}H_{21}N_3,HCl$ Mol. Wt. 291.82

Category : Antihistaminic.

Dose : 50 to 100 mg, three or four times a day as necessary.

Description : White, crystalline powder; odourless; taste, bitter. Slowly darkens on exposure to light.

Solubility : Freely soluble in *water*, in *alcohol* and in *chloroform*; slightly soluble in *acetone*; insoluble in *solvent ether* and in *benzene*.

Standards : Tripelennamine Hydrochloride is the hydrochloride of 2-[*N*-benzyl-*N*-(2-dimethylaminoethyl)-amino] pyridine. It contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of $C_{16}H_{21}N_3,HCl$, calculated with reference to the dried substance.

Identification : (A) To about 50 mg add 2 ml of *sulphuric acid*; a yellow colour is produced which slowly turns to a muddy brown and on diluting the mixure with an equal volume of *water*, the colour changes to greyish white with a green tinge.

(B) Dissolve 0.1 g in 10 ml of *water* containing 1 drop of *0.1N hydrochloric acid*, add an excess of *picric acid solution*, and allow to stand for two hours. Melting range of the precipitate, after washing with *water* and drying is 179° to 184°, with decomposition, Appendix 5.11.

(C) A solution (1 in 20) gives the reactions of *chlorides*, Appendix 3.1.

Melting range : Between 188° and 192°, Appendix 5.11.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for three hours, Appendix 5.8.

Assay : Weigh accurately about 0.3 g and dissolve in a mixture of 10 ml of *glacial acetic acid* and 10 ml of *mercuric acetate solution*. Add two drops of *crystal violet solution* and titrate with *0.1N perchloric acid*. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.01459 g of $C_{16}H_{21}N_3$, HCl.

Storage : Store in well-closed, light-resistant containers.

Tripelennamine Tablets

Category : Antihistamine.

Dose : Tripelennamine Hydrochloride, 50 to 100 mg three or four times a day as necessary.

Usual strengths : 25 mg and 50 mg.

Standards : Tripelennamine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of Tripelennamine Hydrochloride, $C_{16}H_{21}N_3$, HCl.

Identification : Extract a quantity of the finely powdered tablets equivalent to 50 mg of Tripelennamine Hydrochloride with 5 ml portions of *chloroform*. Filter the combined chloroform extracts and evaporate to dryness; the residue complies with the **Identification** test (A) described under Tripelennamine Hydrochloride.

Disintegration : Maximum time, 30 minutes, Appendix 5.6.1.

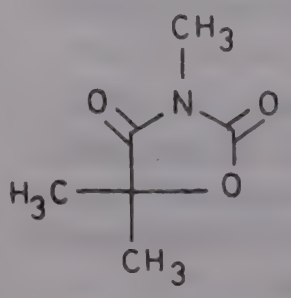
Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 10 mg of Tripelennamine Hydrochloride and shake with 50 ml of *methyl alcohol*. Add sufficient *methyl alcohol* to produce 100.0 ml and mix. Centrifuge, dilute 10.0 ml of the supernatant liquid to 100.0 ml with *methyl alcohol*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 246 nm, Appendix 5.15 A. Calculate the content of $C_{16}H_{21}N_3$, HCl, taking 547 as the E(1 per cent, 1-cm) at the maximum at about 246 nm.

Storage : Store in well-closed containers.

Troxidone

Trimethedione



$C_6H_9NO_3$ Mol. Wt. 143.14

Category : Anticonvulsant.

Dose : For adults, 1 to 2 g daily, in divided doses; for children 0.25 to 0.5 g daily, in divided doses.

Description : White crystalline granules; odour, slightly camphor-like.

Solubility : Soluble in *water*; freely soluble in *alcohol*, in *chloroform* and in *solvent ether*.

Standards : Troxidone is 3,5,5-trimethyloxazolidine-2,4-dione. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_6H_9NO_3$, calculated with reference to the dried substance.

Identification : (A) To 5 ml of a 2 per cent w/v solution add 2 ml of *barium hydroxide solution*; a precipitate is formed immediately.

(B) To 0.5 g add 4 ml of *sodium hydroxide solution*, heat on a water-bath for thirty minutes, and finally evaporate to a volume of about 0.5 ml over a free flame, cool and carefully acidify the residue with *hydrochloric acid*, using *litmus paper* as the indicator; add one drop of *ferric chloride solution* to 10 drops of the solution so prepared; a deep yellow colour is produced.

(C) Place the remainder of the acid solution obtained in **Identification** test (B) in a separator and extract with three quantities, each of 10 ml, of *solvent ether*. Evaporate the *ether*; the residue, on recrystallising from *benzene* and drying over *silica gel* for four hours, melts between 79° and 81°, Appendix 5.11.

Melting range : Between 45° and 47°, Appendix 5.11.

Heavy metals : Not more than 20 parts per million, determined on 1.0 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying over *silica gel* for six hours, Appendix 5.8.

Assay : Dissolve 1 g of *2-phenylethanol* (internal standard) in sufficient *methyl alcohol* to produce 50 ml (solution A). Carry out the method for *gas-liquid chromatography*, Appendix 5.4.1, using the following solutions: (1) to 0.2 g of *troxidone R.S.* add 5 ml of solution A and sufficient *methyl alcohol* to produce 10 ml; (2) treat 0.2 g of the substance being examined in a similar manner but omitting the addition of soluton A; (3) treat 0.2 g of the substance being examined as described under solution (1). The chromatographic procedure may be carried out using (a) a glass column 1.5 m long and 0.4 cm in internal diameter packed with 10 per cent w/w of diethyleneglycol succinate supported on acid-washed, silanised diatomaceous earth (100 to 120 mesh) and maintained at 150°, (b) nitrogen as the carrier gas, and (c) a flame ionisation detector. Calculate the content of $C_6H_9NO_3$, using the declared content of $C_6H_9NO_3$ in *troxidone R.S.*

Storage : Store in tightly-closed containers, in a cool place.

Troxidone Capsules

Trimethadione Capsules

Category : Anticonvulsant.

Dose : Troxidone. For adults, 0.9 g daily, in divided doses, increasing to 1.8 g in accordance with the needs of the patient; for children, 0.3 to 0.6 g daily in divided doses.

Usual strength : 0.3 g.

Standards : Troxidone Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of troxidone, $C_6H_9O_3N$.

Identification : To a quantity of the contents of the capsules equivalent to 1 g of troxidone add 25 ml of *solvent ether* in a stoppered flask for twenty minutes, decant the ether through a filter, and if an insoluble residue remains, digest it with another 10 ml portion of *solvent ether* as before, and filter into the first ether filtrate. Evaporate the ether solutions to dryness, preferably with a current of air, and dry the residue under reduced pressure for two hours; the residue has a melting range of 45° to 47°, Appendix 5.11, and complies with the **Identification** tests (A) to (C) described under Troxidone.

Other requirements : Comply with the requirements stated under Capsules.

Assay : Carry out the **Assay** described under Troxidone, using solution (2) and (3) prepared as follows: Solution (2) is prepared in the same manner as solution (3) but replacing solution A with *methyl alcohol*. Solution (3) is the supernatant liquid obtained by adding 25 ml of solution A to a quantity of the contents of the capsules equivalent to 1.0 g of troxidone, shaking for thirty minutes, adding sufficient *methyl alcohol* to produce 50.0 ml and centrifuging.

Storage : Store in tightly-closed containers.

Old Tuberculin

Category : Diagnostic aid (dermal reactivity indicator).

Dose : By intradermal injection, 0.1 ml of solution containing in each ml 10,100 or 1,000 International Units.

NOTE—Old Tuberculin in its undiluted state is not administered to the patient. It is normally used after dilution.

Description : Transparent, yellow to brown, viscous liquid.

Standards : Old Tuberculin is the heat-concentrated filtrate from a fluid medium on which the human or bovine strain of *Mycobacterium tuberculosis* has been grown.

The organism is grown on a liquid medium containing 5 per cent w/v of Glycerin or any other suitable medium at about 37° for six weeks or more. The growth should be rapid and abundant. The cultures are heated in free-flowing steam for at least one hour. The culture medium, from which the organisms may or may not have been previously separated by filtration, is concentrated by evaporation on a water-bath under vacuum, to about one-tenth of its original volume, clarified by filtration, after the addition of 0.5 per cent w/v of phenol or any other suitable preservative assayed and then diluted. The final sterile product is distributed in sterile glass containers which are then sealed so as to prevent microbial contamination. The final product is free from live *tubercle bacilli*.

It contains not less than 100,000 International Units per ml.

Identification : Inoculate intradermally into suitable sensitised healthy guinea-pigs, weighing not less than 250 g, progressively increasing doses; reactions occur at the points of injection, varying in intensity from blisters with erythema to neurosis.

Freedom from live mycobacteria : Inject intraperitoneally or subcutaneously into two guinea-pigs, weighing not less than 250 g, the deposit centrifuged from 5.0 ml and resuspended in a small volume of *saline solution*. Observe the animals for at least 42 days and then kill them and examine at autopsy. Neither of the animals shows signs of the presence of mycobacterium.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Toxicity : Inject 0.5 ml subcutaneously into two healthy guinea-pigs weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test. No harmful effects are produced within seven days.

Assay : Determine the potency by the *biological assay of old tuberculin*, Appendix 2.31. The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The fiducial limits of error are not less than 75 per cent and not more than 130 per cent of the stated potency.

Storage : Store at a temperature between 2° and 10°, protected from light.

Labelling : The label on the container states (1) the number of International Units per ml; (2) the total volume in the container; (3) the name and proportion of any added substances; (4) the species or strain used; (5) the storage conditions; (6) the expiry date.

Tuberculin Purified Protein Derivative

Tuberculin PPD

Category : Diagnostic aid (dermal reactivity indicator).

Dose : By intradermal injection, 0.1 ml containing 1, 10 or 100 International Units.

Description : Colourless or pale, straw-coloured liquid or cream-coloured powder or pellet.

Standards : Tuberculin Purified Protein Derivative is a preparation made from the heat-treated products of growth and lysis of the tubercle bacillus *(Mycobacterium tuberculosis* or *Mycobacterium bovis)*.

It is prepared from the water-soluble fraction obtained by heating in free-flowing steam and subsequently filtering cultures of the mycobacteria grown in a suitable liquid medium. The protein fraction of the filtrate is separated by precipitation, washed and redissolved. The final sterile product is distributed into sterile glass containers which are then sealed so as to prevent microbial contamination or alternatively it is freeze-dried and the containers subsequently sealed.

The liquid preparation contains 100,000 International Units per ml and the freeze-dried preparation contain 30,000 International Units per mg.

Identification : Complies with the test described under Old Tuberculin.

pH : Between 6.5 and 7.5, determined in the liquid preparation or the reconstituted freeze-dried preparation, Appendix 5.10.

Phenol (if present) : Not more than 0.5 per cent w/v, Appendix 3.3.9.

Toxicity : Inject subcutaneously into two healthy guinea-pigs, weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test, 0.5 ml of a solution containing 100,000 International Units per ml. No harmful effects are produced within seven days.

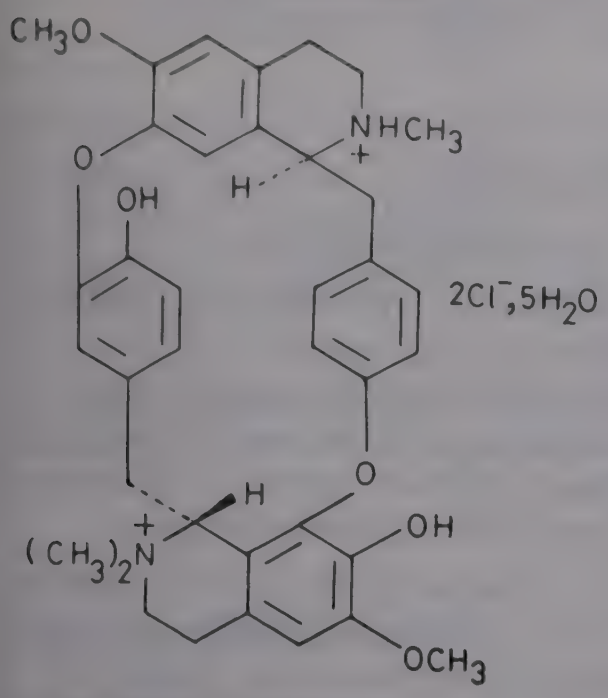
Assay : Determine the potency by the *biological assay of old tuberculin*, Appendix 2.31. The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The fiducial limits of error are not less than 75 per cent and not more than 130 per cent of the stated potency.

Storage : Store at a temperature between 2° and 10°, protected from light.

Labelling : The label on the container states (1) the number of International Units per ml or mg; (2) the total volume in the container (for liquid preparation); (3) the volume and quantity of the reconstituting liquid (for the freeze-dried preparation); (4) the name and proportion of any added substance; (5) the species or strain used; (6) the storage conditions; (7) the expiry date; (8) "Do not inhale" (for the freeze-dried preparation).

Tubocurarine Chloride

d-Tubocurarine Chloride



$C_{37}H_{41}ClN_2O_6,HCl,5H_2O$ Mol. Wt. 771.73

Category : Skeletal muscle relaxant.

Dose : By intramuscular or intravenous injection, 0.1 to 0.3 mg per kg of body weight, not exceeding 25 mg, then 0.025 to 0.1 mg per kg repeated as necessary.

Description : White or yellowish-white, crystalline powder; odourless.

Solubility : Soluble in *water*; sparingly soluble in *alcohol*; practically insoluble in *solvent ether*, in *chloroform* and in *acetone*.

Standards : Tubocurarine Chloride is the pentahydrate of 7′, 12′-dihydroxy-6,6-dimethoxy-2,2′,2′-trimethyltubocurararanium chloride hydrochloride. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{37}H_{41}ClN_2O_6,HCl$, calculated with reference to the anhydrous substance.

Identification : (A) To 1 ml of a 1 per cent w/v solution add 0.15 ml of a 1.3 per cent w/v solution of *ferric chloride hexahydrate*; a green colour is produced which becomes brown on warming on a water-bath.

(B) To 20 ml of a 0.05 per cent w/v solution add 0.2 ml of *sulphuric acid* and 2 ml of a 1.0 per cent w/v solution of *potassium iodate*, mix and warm on a water-bath for thirty minutes; a yellow colour is produced.

(C) It melts at about 270°, with decomposition, Appendix 5.11.

(D) A solution (1 in 100) gives the reactions of *chlorides*, Appendix 3.1.

Specific optical rotation : Between +210° and +218°, determined in a 1.0 per w/v solution three hours after preparation, Appendix 5.12.

pH : Between 4.0 and 6.0, determined in a 1.0 per cent w/v solution, Appendix 5.10.

Chloroform-soluble substances : Not more than 2.0 per cent, determined in the following manner: Dissolve 0.25 g in 150 ml of *water* contained in a separator having a grease-free stopcock. Add 5 ml of a saturated solution of *sodium bicarbonate* and extract with three quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with 10 ml of *water*, filter the chloroform solution into a tared beaker and wash the filter with two successive quantities, each of 5 ml of *chloroform*. Add the washings to the filtrate. Remove the chloroform on a water-bath, dry at 105° for one hour, cool and weight. The residue does not dissolve in 10 ml of *water*, but dissolves in 1 ml of *dilute hydrochloric acid*.

Sulphated ash : Not more than 0.25 per cent, Appendix 3.2.7.

Water : Not more than 12.0 per cent w/w, Appendix 3.3.25.

Assay : Weigh accurately about 25 mg and dissolve in sufficient *water* to produce 500.0 ml. Measure the *extinction* of the resulting solution at the maximum at about 280 nm, Appendix 5.15 A. Calculate the content of $C_{37}H_{41}ClN_2O_6,HCl$ taking 118 as the value of E(1 per cent, 1-cm) at the maximum at about 280 nm.

Storage : Store in tightly-closed containers.

Tubocurarine Injection

Tubocurarine Chloride Injection

Category : Skeletal muscle relaxant.

Dose : Tubocurarine Chloride. By intramuscular or intravenous injection, 0.1 to 0.3 mg per kg of body weight, not exceeding 25 mg, then 0.025 to 0.1 mg per kg repeated as necessary.

Usual strength : 10 mg per ml.

Description : Clear, colourless or faintly coloured solution.

Standards : Tubocurarine Injection is a sterile solution of Tubocurarine Chloride in Water for Injection. It contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of $C_{37}H_{42}Cl_2N_2O_6,5H_2O$. It may contain a suitable buffering agent.

Identification : (A) Dilute 1 ml to 30 ml with *water*. To 1 ml of the solution add 0.5 ml of *mercury nitrate solution*; a cherry-red colour slowly develops.

(B) It gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 3.0 and 6.0, Appendix 5.10.

Optical rotation : Between +0.172° and +0.206° for each mg of tubocurarine chloride $C_{37}H_{42}Cl_2N_2O_6,5H_2O$ per ml stated on the label, Appendix 5.12.

Other requirements : Complies with the requirements stated under Injections.

Assay : Dilute 5.0 ml to 1000.0 ml with *water* and measure the *extinction* of the resulting solution at the maximum at about 280 nm, Appendix 5.15 A. Calculate the content of $C_{37}H_{42}Cl_2N_2O_6,5H_2O$, taking 105 as the E(1 per cent, 1-cm) at the maximum at about 280 nm.

Storage : Store in single-dose or multiple-dose containers.

Typhoid Vaccine

Category : Active immunising agent.

Dose : Prophylactic, by subcutaneous injection, 0.5 ml as initial dose; second dose of 1 ml after an interval of four to six weeks.

Standards : Typhoid Vaccine is a sterile suspension prepared from one or more strains of *Salmonella typhi* that are smooth and have the full complement of O, H and Vi antigens. The bacteria are killed by heat or by a bactericide such as phenol or formaldehyde or by a chemical such as acetone.

It contains not less than 1000 million bacteria *(S. typhi)* per human dose which does not exceed 1 ml.

Description : White or creamy-white turbid liquid, free from clumps.

Identification : It is identified by specific agglutination.

pH : Between 6.8 and 7.4, Appendix 5.10.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Undue toxicity : Complies with the *test for undue toxicity for vaccines and sera*, Appendix 2.3.7.

Phenol (if present) : Not more than 0.5 per cent w/v, Appendix 3.3.9.

Potency : Carry out the *biological assay of typhoid vaccines*, Appendix 2.29.

Storage : Store at a temperature between 2° and 8°. The vaccine must not be frozen.

Labelling : The label on the container states (1) the number of bacteria per human dose; (2) the storage conditions; (3) the date after which the contents are not intended to be used; (4) "Not to be frozen"; (5) Shake well before use; (6) the name and proportion of any added preservative.

Typhus Vaccine

Category : Active immunising agent.

Dose : Prophylactic, by subcutaneous injection, 0.25 to 1.0 ml.

Standards : Typhus Vaccine is a sterile suspension of the killed rickettsiae organisms of a strain or strains of epidemic typhus rickettsiae *(Rickettsia prowazeki)* selected for antigenic efficiency.

The vaccine is prepared by injecting virulent rickettsiae into the yolk sacs of fertile eggs which have been incubated for seven days. Within nine to thirteen days after injection, the yolk sacs are collected under aseptic conditions as soon as practicable and subjected to suitable treatment to liberate the maximum number of rickettsiae. The material is suspended in *saline solution* or any other solution isotonic with blood, to which Formaldehyde Solution has been added so that the concentration of formaldehyde is 0.2 to 0.5 per cent. The suspension which contains 10 to 15 per cent w/w of yolk sac tissue is purified by treatment with Solvent Ether. The aqueous middle layer of the resultant mixture is collected and distributed under aseptic conditions into sterile containers which are then sealed so as to exclude micro-organisms.

The vaccine may also be prepared from the lungs of small rodents in which rickettsial pneumonias have been caused by inhalation of massive doses of virulent rickettsiae, or from the peritoneal cavities of gerbils which have had intraperitoneal injections of rickettsiae.

The vaccine may contain a suitable preservative.

Description : Slightly turbid liquid; a white deposit of the rickettsiae may separate on standing, but it can be redistributed on shaking.

Identification : It specifically protects laboratory animals against epidemic and murine typhus.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Undue toxicity : Complies with the *test for undue toxicity for vaccines and sera*, Appendix 2.37, when injected subcutaneously or intravenously or intraperitoneally into mice or guinea-pigs.

Potency : Carry out the *biological assay of typhus vaccine*, Appendix 2.29. The vaccine passes the test if the serum of immunised guinea-pigs, when diluted 1 to 32, protects mice against the toxin of epidemic typhus rickettsiae.

Storage : Store at a temperature between 2° and 8°.

Labelling : The label on the container states (1) the nature of the preparation, i.e. whether it has been prepared in eggs, rodent's lung or otherwise, and whether it has been purified; (2) "Shake well before use"; (3) the storage conditions; (4) the date after which it is not intended to be used; and (5) the name and proportion of any added preservatives.

Undecylenic Acid

Undecenoic Acid

$CH_2=CH(CH_2)_8COOH$

CH_2:CH.$(CH_2)_8$,COOH Mol. Wt. 184.28

Category : Antifungal (topical).

Description : Colourless to pale yellowish-brown clear liquid or white to yellowish-white, crystalline mass; odour, characteristic.

Solubility : Practically insoluble in *water*, miscible with *alcohol*, with *chloroform*, with *solvent ether*, and with fixed and volatile oils.

Standards : Undecylenic Acid consists mainly of 10-undecenoic acid. It contains not less than 96.0 per cent and not more than the equivalent of 101.0 per cent of $C_{11}H_{20}O_2$.

Identification : (A) To 1 g add *potassium permanganate solution* dropwise with shaking; the colour of the permanganate is discharged.

(B) Heat 3 g with 3 ml of freshly distilled *aniline* in a long test-tube for ten minutes so that the condensate remains just below the mouth of the tube. Cool, add 10 ml of *alcohol* and 10 ml of *solvent ether*, wash the mixture with four quantities, each of 20 ml, of *water*, and reject the washings. Evaporate the ether, add 5 mg of *decolorising charcoal*, filter, and evaporate the filtrate to dryness; the residue, after recrystallisation from *alcohol (70 per cent)*, melts at about 66°, Appendix 5.11.

Congealing range : Between 21° and 24°, Appendix 5.5.

Refractive index : Between 1.447 and 1.450, Appendix 5.14.

Iodine value : Between 131 and 140, Appendix 3.3.18.

Water-soluble acids : Shake 5 g with 5 ml of warm *water*, allow to separate, and filter the aqueous layer through a moistened filter paper; titration with *0.1N sodium hydroxide* using *methyl orange solution* as indicator; not more than 0.1 ml of *0.1N sodium hydroxide* is required.

Neutral fats and mineral oils : Boil 1 g in a large flask with 10 ml of *N sodium carbonate* and 20 ml of *water*; the solution while hot is clear or at most opalescent.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method B, Appendix 3.2.4.

Sulphated ash : Not more than 0.15 per cent, Appendix 3.2.7.

Assay : Weigh accurately about 3 g and dissolve in 10 ml of *alcohol*, previously neutralised to *phenolphthalein solution* and titrate with *0.5N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.5N sodium hydroxide* is equivalent to 0.09214 g of $C_{11}H_{20}O_2$.

Storage : Store in tightly-closed, light-resistant containers.

Urea

$H_2N.CO.NH_2$

CH_4N_2O Mol. Wt. 60.06

Category : Diuretic.

Dose : 5 to 15 g.

Description : Colourless, transparent, prismatic crystals or white crystalline powder; odourless or almost odourless but may gradually develop a slight odour of ammonia upon long standing; taste, cooling and saline, slightly hygroscopic.

Solubility : Freely soluble in *water* and in boiling *alcohol*; soluble in *alcohol*; practically insoluble in *chloroform* and in *solvent ether*.

Standards : Urea is the diamide of carbonic acid. It contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of CH_4N_2O.

Identification : (A) Heat 0.5 g in a test-tube; it liquefies, and ammonia is evolved which is recognised by its characteristic odour. Heat further until the liquid is turbid, cool and dissolve in 10 ml of *water*. Add 1 ml of a 10 per cent w/v solution of *sodium hydroxide* and one drop of *copper sulphate solution*, a reddish-violet colour is produced.

(B) Dissolve 0.1 g in 1 ml of *water* and add 1 ml of *nitric acid*; a white, crystalline precipitate is produced.

Melting range : Between 132° and 135°, Appendix 5.11.

Alcohol-insoluble matter : Not more than 0.04 per

cent, determined by the following method: Dissolve 5 g in 50 ml of warm *alcohol*, filter through a tared filter, wash the filter with 20 ml of warm *alcohol* and dry at 105° for one hour.

Chloride : 5.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Sulphate : 6.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Heavy metals : Not more than 20 parts per million, determined by Method A on 1.0 g dissolved in 20 ml of *water* and 5 ml of *0.1N hydrochloric acid*, Appendix 3.2.4.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Assay : Weigh accurately about 0.17 g and transfer to a 300-ml long necked flask. Add 25 ml of *water*, 2 ml of a 3 per cent w/v solution of *copper sulphate* and 8 ml of *sulphuric acid*. Heat gently for fifteen minutes so that copious fumes are evolved, cool, and slowly add 100 ml of *water* and 0.2 g of *granulated zinc*. Connect the flask to an ammonia distillation apparatus. The delivery tube of the apparatus should dip in 50 ml of a 2 per cent w/v solution of *boric acid*. Heat the flask and when the air is driven out, add slowly 75 ml of *sodium hydroxide solution*. Distil and collect the ammonia. Titrate the distillate with *0.2N hydrochloric acid*, using *methyl red solution* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of ammonia evolved from the urea. Each ml of *0.2N hydrochloric acid* is equivalent to 0.006006 g of CH_4N_2O.

Storage : Store in well-closed containers.

Vaccines

Vaccines are preparations of antigenic substances that are administered for the purpose of inducing in the recipient a specific and active immunity against the infecting agent or toxin produced by it. They are prepared from bacteria, viruses, rickettsiae or toxins.

Vaccines may contain living micro-organisms suitably treated to attenuate their virulence but retain their antigenic potency or they may consist of pathogenic organisms which have been killed or inactivated. Some vaccines consist of antigenic fractions or substances produced by the same pathogenic organisms but rendered harmless whilst retaining their antigenic efficiency. Vaccines may be prepared from one species only or from a mixture of two or more species.

Vaccines may be prepared by the method described in the individual monographs or by the general methods given below or in any other manner provided the identity of the antigens is maintained and the vaccines are free from microbial contamination and extraneous agents.

Suitable adjuvants may be added during the preparation but penicillin or streptomycin may not be added at any stage of manufacture or in the final vaccine. A suitable bactericide may be added to sterile and inactivated vaccines.

The final products are distributed aseptically into sterile containers which are than sealed to exclude extraneous micro-organisms. Unless otherwise indicated in the monograph, the final vaccine may be filled into single-dose or multiple-dose containers, but vaccines in multi-dose containers must invariably contain a bactericide.

Bacterial Vaccines : Bacterial Vaccines are either sterile suspensions of live or killed bacteria or are sterile extracts of derivatives of bacteria. They may be simple vaccines prepared from one species or may be mixed vaccines prepared by blending two or more simple vaccines from different species or varieties.

Bacterial Vaccines may be prepared from cultures grown on suitable solid or liquid media. The whole culture or parts of it may be used in preparing the vaccine. The identity, antigenic potency and purity of each bacteria culture must be carefully controlled.

Vaccine containing killed organisms may be prepared by killing the organisms by chemical or physical means provided the antigenic potency of the vaccine is preserved. Vaccine containing living bacteria may be prepared from strains which are a virulent for man but which stimulate the production of antibodies active against pathogenic strains of the same species. The final vaccines must be free from any substance known to cause toxic, allergic or other undesirable immunological reactions in man.

Bacterial Vaccines are suspensions of varying degrees of opacity in colourless or slightly coloured liquids or they may be freeze-dried. They may be standardised in terms of international opacity units or, where appropriate by numbers of living or killed bacteria determined by direct cell count or viable count.

Bacterial toxoids : Bacterial toxoids are toxins or material derived therefrom, the toxicity of which has been reduced to a very low level or completely eliminated by chemical or physical means without destroying their immunising potency. The toxins are obtained from selected strains of specific micro-organisms, grown in media free from ingredients known to cause toxic, allergic or other undesirable immunological reactions in man.

Toxoids produced by the action of formaldehyde are known as formal toxoids.

Bacterial toxoids may be liquid or may be prepared by adsorbing on mineral carriers such as aluminium phosphate, aluminium hydroxide or any other adsorbent; the adsorbed product may be separated, washed, and suspended in a saline or other appropriate solution isotonic with blood.

Bacterial toxoids are clear or slightly opalescent liquids, colourless or slightly yellow. Adsorbed vaccine may be white or greyish-white suspensions or pale-yellow liquid with a sediment at the bottom of the container. Freeze-dried preparations are greyish-white or yellowish-white powders or pellets.

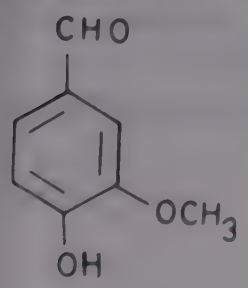
Viral and rickettsial vaccines : Viral and rickettsial vaccines are suspensions of viruses or rickettsiae and are prepared form infected tissue or blood obtained from artificially infected animals, from cultures in fertile eggs, or from cell or tissue cultures. Viral vaccines may be live or killed and they may be freeze-dried. Live vaccines are usually prepared using attenuated strains of the specific organism. Killed vaccines may be inactivated by suitable chemical or physical means.

Mixed vaccines : Mixed vaccines are mixtures of two or more vaccines. A suitable antibacterial substance may be added to inactivated or live viral and rickettsial vaccines, provided that it has no action against the specific organisms.

Storage of vaccines : Vaccines must be protected from light and stored at a temperature between 2° and 8°, unless otherwise specified in the individual monograph. Freeze-dried preparations must be stored at a temperature below 20°. At higher temperatures vaccines deteriorate rapidly. Unless otherwise specified, liquid vaccines should not be allowed to freeze.

NOTE – The statements given in this general monograph are intended to be read in conjunction with the monographs on the individual vaccines in the Pharmacopoeia, which refer to preparations for human use; they do not necessarily apply for use in Veterinary medicines.

Vanillin



$C_8H_8O_3$ Mol. Wt. 152.15

Category : Pharmaceutical aid (flavour).

Description : White or cream-coloured crystalline needles or powder; odour, characteristic of vanillin.

Solubility : Slightly soluble in *water*; freely soluble in *alcohol*, in *chloroform*, in *solvent ether* and in solutions of alkali hydroxides; soluble in *glycerin* and in hot *water*.

Standards : Vanillin is 4-hydroxy-3-methoxy-benzaldehyde. It may be obtained from any species of *Vanilla* (Fam. Orchidaceae) or prepared synthetically. It contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of $C_8H_8O_3$, calculated with reference to the dried substance.

Identification : (A) To 1 ml of a saturated solution in *water*, add 1 ml of *lead acetate solution*; a white precipitate is formed which is soluble in hot *water* from which it separates in scales on cooling.

(B) To 10 ml of a saturated solution in *water*, add 0.2 ml of *ferric chloride test-solution*; a blue colour is produced. Heat at 80° for three minutes; the solution becomes brown. Cool; a white or almost white precipitate is formed.

Melting range : Between 81° and 83°, Appendix 5.11.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 1.0 per cent, determined on 1.0 g by drying over *silica gel* for four hours, Appendix 5.8.

Assay : Weigh accurately about 0.1 g and dissolve in sufficient *methyl alcohol* to produce 250.0 ml. Dilute 2.0 ml to 100.0 ml with *methyl alcohol* and mix. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 308 nm, Appendix 5.15 A. Calculate the content of $C_8H_8O_3$, from the *extinction* obtained by carrying out the Assay simultaneously on *vanillin R.S.* and from the declared content of $C_8H_8O_3$ in the *vanillin R.S.*

Storage : Store in tightly-closed, light-resistant containers.

Vasopressin Injection

Category : Posterior pituitary hormone; antidiuretic.

Dose : By subcutaneous or intramuscular injection, 0.1 to 1.0 ml (2 to 20 Units).

Usual strength : 20 Units per ml.

Description : Clear, colourless or practically colourless liquid with a faint characteristic odour.

Standards : Vasopressin Injection is a sterile aqueous solution containing the water-soluble pressor principle obtained from the posterior lobe of the pituitary of healthy oxen or other mammals or by synthesis. It contains a pressor activity equivalent to not less than 90 per cent and not more than 110 per cent of the stated number of Units of vasopressor activity.

Identification : (A) Inject into the vein of a mammal, anaesthetised by a general anaesthetic or by destruction of the brain; it causes a rise of blood pressure.

(B) Inject under the skin of a mammal and at the same time administer a volume of *water* by mouth; it causes a delay in the excretion of the water.

pH : Between 2.5 and 4.5, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

Oxytocin activity : Not more than 1.2 Units, assayed by the *biological assay of oxytocin injection, rat uterus method*, Appendix 2.12.

Assay : Carry out the *biological assay of vasopressin injection*, Appendix 2.13.

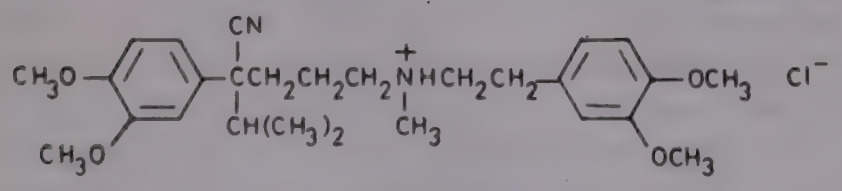
The fiducial limits of error are not less than 80 per cent and not more than 125 per cent of the stated potency.

Storage : Store in single-dose or multiple-dose containers at a temperature between 2° and 10°.

Labelling : The label on the container states (1) the vasopressor activity as the number of Units per ml; (2) either the animal source of the vasopressin or that it is synthetic; (3) the storage conditions.

Verapamil Hydrochloride

Verapamil Chloride, Iproveratril Hydrochloride



$C_{27}H_{38}N_2O_4,HCl$ Mol. Wt. 491.07

Category : Peripheral vasodilator.

Dose : 40 to 80 mg.

Description : White or almost white, crystalline powder; almost odourless; taste, bitter.

Solubility : Freely soluble in *chloroform*; soluble in *water*; slightly soluble in *alcohol*; practically insoluble in *solvent ether*.

Standards : Verapamil Hydrochloride is *N*[4-cyano-5-methyl-4-(3,4-dimethoxyphenyl)hexyl]-*N*-[2-(3,4-dimethoxyphenyl) ethyl] methylammonium chloride. It contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $C_{27}H_{38}N_2O_4.HCl$, calculated with reference to the dried substance.

Identification : (A) To 2 ml of a 1.5 per cent w/v solution add 0.5 ml of *dilute nitric acid*; a white precipitate is produced.

(B) To 2 ml of a 1.5 per cent w/v solution, add 0.2 ml of a 5 per cent w/v solution of *mercuric chloride*; a white precipitate is produced.

(C) To 2 ml of a 1.5 per cent w/v solution, add 0.5 ml of *bromine solution*; a yellow precipitate is produced.

(D) The light absorption, in the range 230 to 350 nm, of a 1-cm layer of a 0.001 per cent w/v solution in *0.1N hydrochloric acid* exhibits maxima only at 229 nm and 278 nm; *extinction* at 229 nm, about 0.3, at 278 nm, about 0.12, Appendix 5.15 A.

Melting range : Between 140° and 144°, Appendix 5.11.

pH : Between 4.5 and 6.5, determined in a 5.0 per cent w/v solution, Appendix 5.10.

Clarity and colour of solution : A 5.0 per cent w/v solution is clear and almost colourless.

Readily carbonisable substances : Dissolve 0.1 g in 5 ml of *sulphuric acid* (94.5 per cent to 95.5 per cent w/v of H_2SO_4); the solution is not more coloured than 5 ml of a mixture of 0.5 ml of *cobalt chloride C.S.*, 1.6 ml of *ferric chloride C.S.*, 0.5 ml of *copper sulphate C.S.* and 47.4 ml of a 1.0 per cent w/v solution of *hydrochloric acid*.

Sulphated ash : Not more than 0.1 per cent, Appendix 3.2.7.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for two hours, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and dissolve in 40 ml of *glacial acetic acid*. Add 10 ml of *mercuric acetate solution*, 0.1 ml of *crystal-violet solution* and titrate with *0.1N perchloric acid* to a blue-green end-point. Perform a blank determination and make any necessary correction. Each ml of *0.1N perchloric acid* is equivalent to 0.04911 g of $C_{27}H_{38}N_2O_4,HCl$.

Storage : Store in well-closed, light-resistant containers.

Verapamil Injection

Category : Peripheral vasodilator.

Dose : Verapamil Hydrochloride. By slow intravenous injection, 5 mg.

Usual strength : 2.5 mg per ml.

Standards : Verapamil Injection is a sterile solution of Verapamil Hydrochloride in Water for Injection. It contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{27}H_{38}N_2O_4$,HCl.

Description : Clear, colourless solution.

Identification : (A) To a volume equivalent to 5 mg of Verapamil Hydrochloride add 0.2 ml of a 5 per cent w/v solution of *mercuric chloride*; a white precipitate is produced.

(B) To a volume equivalent to 5 mg of Verapamil Hydrochloride add 0.5 ml of *bromine solution*; a yellow precipitate is produced.

(C) Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel GF 254* as the coating substance and a mixture of 68 volumes of *n-hexane*, 30 volumes of *isopropyl alcohol* and 2 volumes of *strong ammonia solution* as the mobile phase. Apply separately to the plate 20 µl of each of the following solutions: (1) evaporate a suitable volume to dryness in a current of *nitrogen* using the minimum of heat; dissolve the residue as completely as possible in sufficient *methyl alcohol* to produce a solution containing 0.4 per cent w/v of Verapamil Hydrochloride; (2) a 0.4 per cent w/v solution of *verapamil hydrochloride R.S.* in *methyl alcohol*. After removal of the plate, allow it to dry in air and examine under an ultra-violet lamp having a maximum output at about 254 nm. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

pH : Between 4.5 and 6.0, Appendix 5.10.

Other requirements : Complies with the requirements stated under Injections.

Assay : Measure accurately a volume equivalent to 5 mg of Verapamil Hydrochloride, add 2.0 ml of *N hydrochloric acid* and dilute to 200.0 ml with *water*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 278 nm, Appendix 5.15 A, using *0.1 N hydrochloric acid* as a blank. Calculate the content of $C_{27}H_{38}N_2O_4$,HCl, taking 118 as the value of E(1 per cent, 1-cm) at the maximum at about 378 nm.

Storage : Store in a single-dose, light-resistant containers.

Verapamil Tablets

Category : Peripheral vasodilator.

Dose : Verapamil Hydrochloride, 120 to 360 mg daily, in divided doses.

Usual strengths : 40 mg; 80 mg; 120 mg.

Standards : Verapamil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Verapamil Hydrochloride, $C_{27}H_{38}N_2O_4$,HCl. The tablets may be coated.

Identification : (A) Extract a quantity of the powdered tablets equivalent to 40 mg of Verapamil Hydrochloride with *chloroform*, filter and evaporate to dryness; the residue complies with **Identification** tests (A) and (B) described under Verapamil Injection.

(B) Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel GF 254* as the coating substance and a mixture of 68 volumes of *n-hexane*, 30 volumes of *isopropyl alcohol* and 2 volumes of *strong ammonia solution* as the mobile phase. Apply separately to the plate 20 µl of each of the following two solutions: (1) extract a quantity of the powdered tablets equivalent to 40 mg of Verapamil Hydrochloride with *methyl alcohol*, filter and evaporate to dryness; dissolve the residue in 10 ml of *methyl alcohol*, (2) a 0.4 per cent w/v solution of *verapamil hydrochloride R.S.* in *methyl alcohol*. After removal of the plate, allow it to dry in air and examine under an ultra-violet lamp having a maximum output at about 254 nm. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

Other requirements : Comply with the requirements stated under Tablets.

Assay : Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to 40 mg of Verapamil Hydrochloride and shake vigorously for ten minutes with a mixture of 20 ml of *N hydrochloric acid* and 150 ml of *water*. Dilute to 200.0 ml with *water* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water*. Measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 278 nm, Appendix 5.15 A. Calculate the content of $C_{27}H_{38}N_2O_4$,HCl, taking 118 as the value of E(1 per cent, 1-cm) at the maximum at about 278 nm.

Storage : Store in tightly-closed containers.

Viper Venom

Daboia Venom

Category : Local haemostatic.

Dose : By intramuscular injection, initial dose, 1 to 3 Mouse Units. Subsequent doses, 5 to 25 Mouse Units or more in gradually increasing doses.

Description : Almost white or very light yellow, dry powder.

Solubility : Dissolves in *water* to give a clear solution with some insoluble residue.

Standards : Viper Venom is the dried secretion obtained from the poison glands of *Viperae russelli* and other species of *Viperae* (Fam. Viperidae). Immediately after extraction, the poisonous secretion is dried from the frozen state. The dried venom is pooled, mixed, dissolved in ice-cold Water for Injection and then filtered through a bacteria-proof filter to give a stock solution. Further dilutions of the stock solution are made with Water for Injection under aseptic conditions to give solutions with the required number of Mouse Units per ml. These solutions are then distributed in single doses into sterile glass containers, dried from the frozen state and sealed in vacuum. It contains not less than 50 Mouse Units per mg.

Identification : (A) It produces almost immediate coagulation of blood and citrated human plasma.

(B) Mix the soluble fraction from at least 0.6 mg with 1 ml of *polyvalent antisnake venom serum*, and incubate the mixture for half-an-hour at 37° Inject 0.5 ml of the mixture intravenously into a group of mice weighing between 17 and 20 g; no animal dies during twenty-four hours of observation.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Assay : Carry out the *biological assay of snake venom*, Appendix 2.33 and express the result in terms of number of Mouse Units per mg.

Storage : Store in single-dose, light resistant containers.

Labelling : The label on the container states (1) the number of Mouse Units per container; (2) the volume of Water for Injection to be used for reconstitution.

Vitamin A

Category : Antixerophthalmic vitamin.

Dose : Prophylactic, 5000 Units of Vitamin A activity, daily; Therapeutic, 10,000 to 200,000 Units of Vitamin A activity, daily.

Solubility : In oily form, insoluble in *water*; slightly soluble in *chloroform*, in *solvent ether* and in *liquid paraffin*.

In solid form, may be dispersible in warm *water*.

Description : *In oily form* – Light yellow to yellowish-red oil or a mixture of oil and crystalline material which when warmed yields a homogeneous light yellow to yellowish-red oil; odour, faint and characteristic.

In solid form – It has the appearance of any diluent that has been added. Odour, faint but not rancid.

Standards : Vitamin A consists of an ester or a mixture of esters (usually the acetate, palmitate or propionate) of Vitamin A alcohol (retinol) $C_{20}H_{30}O$, prepared by synthesis. It may be diluted with edible vegetable oils or it may be incorporated in solid, edible carriers or excipients. It may contain suitable preservatives, dispersants and anti-oxidants. It contains in 1 g not less than 300,000 *Units of Vitamin A activity* and not less than 95.0 per cent and not more than the equivalent of 110.0 per cent of the stated number of *Units of Vitamin A activity*.

Identification : (A) Dissolve a quantity equivalent to 10 to 15 Units in 1 ml of *chloroform* and add 5 ml of *antimony trichloride solution*; a transient blue colour is produced immediately.

(B) Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 80 volumes of *cyclohexane* and 20 volumes of *solvent ether* as the mobile phase. Apply separately to the plate 2 µl of each of four solutions. Prepare solution (1) in the following manner. If Vitamin A is in oily form, dissolve a quantity equivalent to 50 Units of Vitamin A in sufficient *chloroform* to produce 10 ml. If the Vitamin A is in solid form, weigh a quantity equivalent to 500 Units of Vitamin A, transfer to a separator, add 75 ml of *water*, shake vigorously for one minute, extract with 10 ml of *chloroform* by shaking for one minute and centrifuge the chloroform extract. Solutions (2), (3) and (4) are prepared by dissolving sufficient quantities of *retinyl acetate R.S.*, *retinyl palmitate R.S.* and *retinyl propionate R.S.*, respectively in *chloroform* to produce solutions containing about 5 Units of Vitamin A per ml. Develop the chromatogram immediately without evaporating the solvent. After removal of the plate, allow it to dry in air and spray with *antimony trichloride solution*. The principal spot or spots in the chromatogram obtained with solution (1) corresponds to one or more of the spots in the chromatogram obtained with solutions (2), (3) or (4).

Assay : Carry out the *assay of vitamin A*, Appendix

3.3.24.4, using Method A for Vitamin A in liquid form and Method B for Vitamin A in solid form.

Vitamin A diluted with edible oils (oily form) complies with the following additional tests:

Acid value : Not more than 2.0, Appendix 3.3.15.

Peroxides : Add 0.3 g to 25.0 ml of a mixture of 4 volumes of *methyl alcohol* and 6 volumes of *benzene* (solution A). Mix in a test-tube, in the following order: 0.3 ml of a 1.8 per cent w/v solution of *ammonium thiocyanate*, 10.0 ml of *methyl alcohol*, 0.3 ml of *acid ferrous sulphate solution* and 15.0 ml of *benzene* and add 1.0 ml of solution A. The colour produced after five minutes is not more intense than that obtained by repeating the operation replacing solution A with a solution prepared by adding to 1.0 ml of a 27.0 per cent w/v solution of *ferric chloride hexahydrate*, 99 ml of a mixture of 4 volumes of *methyl alcohol* and 6 volumes of *benzene* and diluting 2.0 ml of this solution to 100 ml with the same solvent mixture.

Storage : Store in tightly-closed, light-resistant containers, preferably in an atmosphere of an inert gas and in a cold place.

Labelling : The label on the container states (1) the form in which the vitamin is present; (2) the number of Units of Vitamin A activity in 1 g; (3) the storage conditions; (4) the method of restoring the solution if partial crystallisation has occurred.

Vitamins A and D Capsules

Category : Antixerophthalmic and antirachitic vitamins.

Dose : Prophylactic, 1600 to 2500 Units of Vitamin A and 100 to 200 Units of Vitamin D once a day; Therapeutic, 5000 to 10,000 Units of Vitamin A and 400 to 1000 Units of Vitamin D once a day.

Standards : Vitamins A and D Capsules contain Vitamin A and a source of Vitamin D such as Ergocalciferol or Cholecalciferol dissolved in an edible vegetable oil. They contain not less than 95.0 per cent of the stated amounts of Vitamins A and D.

Assay : (1) *For Vitamin A* – Weigh accurately a portion of the mixed contents of 20 capsules, equivalent to about 500 Units of Vitamin A and carry out the *assay of Vitamin A*, Appendix 3.3.24.4, using Method A, and express the results in Units of Vitamin A per capsule.

(2) *For Vitamin D* – Carry out the *assay of Vitamin D*, Appendix 3.3.24.5, and express the results in Units of Vitamin D per capsule.

Other requirements : Comply with the requirements stated under Capsules.

Storage : Store in tightly-closed, light-resistant containers, in a cool place.

Labelling : The label on the container states (1) the number of Units of Vitamin A and Vitamin D per capsule; (2) the date after which the contents are not intended to be used; (3) the storage conditions.

Concentrated Vitamin A Solution

Category : Antixerophthalmic vitamin.

Dose : 2500 to 25,000 Units daily.

Description : Pale-yellow or brownish-yellow, oily liquid; odour, faint but not rancid; taste, bland.

Standards : Concentrated Vitamin A Solution is a solution of Vitamin A or of a source of Vitamin A such as a suitable fish-liver oil or blend of such oils, in an edible vegetable oil. It contains not less than 50,000 *Units of Vitamin A activity* per g and not less than 95.0 per cent of the stated number of *Units of Vitamin A activity*.

Identification : Dissolve a quantity equivalent to about 20 Units of Vitamin A activity in 1 ml of *chloroform* and add 10 ml of *antimony trichloride solution*; a transient blue colour appears at once.

Acid value : Not more than 2.5, Appendix 3.3.15.

Assay : Carry out the *assay of Vitamin A, Method A*, Appendix 3.3.24.4..

Storage : Store in well-filled, tightly-closed, light-resistant containers, in a cool place.

Labelling : The label on the container states (1) the number of Units of Vitamin A activity per g; (2) the date after which the contents are not intended to be used; (3) the storage conditions.

Concentrated Vitamin D Solution

Category : Antirachitic vitamin.

Dose : Prophylactic, 400 to 1000 Units daily; Therapeutic, 5000 to 50,000 Units daily.

Description : Pale-yellow or yellow, oily liquid; odour, faint but not rancid; taste, bland.

Standards : Concentrated Vitamin D Solution is a solution of Ergocalciferol or Cholecalciferol in an edible vegetable oil. It contains not less than 10,000 *Units of Vitamin D activity* per g and not less than 90.0 per cent of the stated number of *Units of Vitamin D activity*.

Identification : Dissolve a quantity equivalent to about 1000 Units of Vitamin D activity in 1 ml of *chloroform* and add 10 ml of *antimony trichloride solution*; a pinkish-red colour appears at once.

Acid value : Not more than 2.5, Appendix 3.3.15.

Assay : Carry out the *assay of Vitamin D*, Appendix 3.3.24.5.

Storage : Store in well-filled, tightly-closed, light-resistant containers, in a cool place.

Labelling : The label on the container states (1) the number of Units of Vitamin D activity per g; (2) the date after which the contents are not intended to be used; (3) the storage conditions.

Concentrated Vitamins A and D Solution

Category : Antixerophthalmic and antirachitic vitamins.

Dose : 2500 to 25,000 Units of Vitamin A and 250 to 2500 Units of Vitamin D daily.

Description : Pale-yellow or yellow, oily liquid; odour, faint but not rancid; taste, bland.

Standards : Concentrated Vitamins A and D Solution is a solution of Vitamin A and a source of Vitamin D such as Ergocalciferol or Cholecalciferol in an edible vegetable oil It contains not less than 50,000 *Units of Vitamin A activity* and 5000 *Units of Vitamin D activity* per g and not less than 90.0 per cent of the stated amounts of *Vitamin A activity* and *Vitamin D activity*.

Acid value : Not more than 2.5, Appendix 3.3.15.

Assay : (1) *For Vitamin A activity*—Carry out the *assay of Vitamin A, Method A*, Appendix 3.3.24.4.

(2) *For Vitamin D activity*—Carry out the *assay of Vitamin D*, Appendix 3.3.24.5.

Storage : Store in well-filled, tightly-closed, light-resistant containers, in a cool place.

Labelling : The label on the container states (1) the number of Units of Vitamin A activity and Vitamin D activity per g; (2) the date after which the contents are not intended to be used; (3) the storage conditions.

Purified Water

H_2O Mol. Wt. 18.02

Category : Pharmaceutical aid (solvent).

Description : Clear, colourless liquid; odourless; tasteless.

Standards : Purified Water is prepared from potable water by distillation, ion-exchange treatment, reverse osmosis or any other suitable process. It contains no added substances.

pH : Between 4.5 and 7.0 determined in a solution prepared by adding 0.3 ml of a saturated solution of *potassium chloride* to 100 ml of the liquid being examined, Appendix 5.10.

Carbon dioxide : To 25 ml add 25 ml of *calcium hydroxide solution*; no turbidity is produced.

Chloride : To 10 ml add 1 ml of *dilute nitric acid* and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Sulphate : To 10 ml add 0.1 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride solution*; the solution remains clear for an hour.

Nitrates and nitrites : To 50 ml add 18 ml of *acetic acid* and 2 ml of *naphthylamine-sulphanilic acid reagent*. Add 0.12 g of *zinc reducing mixture* and shake several times. No pink colour develops within fifteen minutes.

Ammonium : To 20 ml add 1 ml of *alkaline potassium mercuri-iodide solution* and after five minutes view in a *Nessler cylinder* placed on a white tile; the colour is not more intense than that given on adding 1 ml of *alkaline potassium mercuri-iodide solution* to a solution containing 2.5 ml of *dilute ammonium chloride solution (Nessler's)* and 7.5 ml of the liquid being examined.

Calcium : To 10 ml add 0.2 ml of *dilute ammonia solution* and 0.2 ml of *ammonium oxalate solution*; the solution remains clear for an hour.

Heavy metals : Adjust the pH of 40 ml to between 3.0 and 4.0 with *dilute acetic acid*, add 10 ml of freshly prepared *hydrogen sulphide solution* and allow to stand for ten minutes; the colour of the solution is not more than that of a mixture of 50 ml of the liquid being examined and the same amount of *dilute acetic acid* added to the sample.

Oxidisable matter : To 100 ml add 10 ml of *dilute sulphuric acid* and 0.1 ml of *0.1 N potassium permanganate* and boil for five minutes. The solution remains faintly pink.

Total solids : Not more than 0.001 per cent w/v, determined on 100 ml by evaporating on a water-bath and drying in an oven at 105° for one hour.

Storage : Store in tightly-closed containers.

NOTE – When distilled water is prescribed or demanded, Purified Water shall be dispensed or supplied.

Water for Injection

H_2O Mol. Wt. 18.02

Category : Pharmaceutical aid (solvent).

Description : Clear, colourless liquid; odourless; tasteless.

Standards : Water for Injection is distilled water, free from pyrogens. It is obtained by distilling potable water, Purified Water, or distilled water from a neutral glass or suitable metal still fitted with an efficient device for preventing the entrainment of droplets; the first portion of the distillate is rejected and the remainder is collected in suitable containers, previously rinsed with freshly distilled water and closed so as to avoid contamination. It contains no added substances.

CAUTION – Water for Injection intended for parenteral solutions that are prepared under aseptic conditions and are not sterilised by filtration or in the final container should be first rendered sterile and then protected from microbial contamination.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using per kg of the rabbit's weight, 10 ml rendered isotonic by the addition of 0.9 g of pyrogen-free *sodium chloride* for each 100 ml.

Other requirements : Complies with all the requirements stated under Purified Water.

Storage : Store in tightly-closed containers.

Sterile Water for Injection

Category : Pharmaceutical aid (solvent).

Description : Clear, colourless liquid; odourless.

Standards : Sterile Water for Injection is Water for Injection sterilised and suitably packed. It contains no antimicrobial agent or other added substances.

pH : Between 4.5 and 7.5, determined in a solution prepared by adding 0.3 ml of a saturated solution of *potassium chloride* to 100 ml of Sterile Water for Injection, Appendix 5.10.

Oxidisable substances : To 100 ml add 10 ml of *dilute sulphuric acid* and 0.2 ml of *0.1N potassium permanganate* for Sterile Water for Injection in containers of upto 50-ml size, or 0.4 ml of *0.1N potassium permanganate* for larger sizes and boil for five minutes. The pink colour does not completely disappear.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Pyrogens : Complies with the *test for pyrogens*, Appendix 2.36, using per kg of the rabbit's weight, 10 ml rendered isotonic by the addition of 0.9 g of pyrogen-free *sodium chloride* for each 100 ml.

Total solids : Not more than 0.004 per cent w/v for Sterile Water for Injection in glass containers upto and including 30-ml size; not more than 0.003 per cent w/v from 30-ml upto and including 100-ml size; not more than 0.002 per cent w/v for larger sizes, determined on 100 ml by evaporating on a water-bath and drying the residue in an oven at 105°, for one hour.

Other requirements : Complies with the requirements of the tests for **Carbon dioxide, Chloride, Sulphate, Nitrates and nitrites, Ammonium, Calcium, and, Heavy metals,** stated under Purified Water.

Storage : Store in single-dose containers of not larger than one litre size.

Wool Fat

Anhydrous Lanolin

Category : Pharmaceutical aid (absorbent ointment base).

Description : Pale-yellow substance with the consistency of an ointment; odour, characteristic. When melted, it gives a clear or almost clear yellow liquid.

Solubility : Practically insoluble in *water*; sparingly soluble in *alcohol*; freely soluble in *solvent ether*, and in *chloroform*.

Standards : Wool Fat is the purified, anhydrous, fat-like substance obtained from the wool of sheep.

Identification : (A) Dissolve 0.5 g in 5 ml of *chloroform*, add 1 ml of *acetic anhydride* and 0.1 ml of *sulphuric acid*; an emerald-green colour is produced.

(B) Dissolve 50 mg in 5 ml of *chloroform*, add 5 ml of *sulphuric acid* and shake; a red colour develops and a strong green fluorescence appears in the lower layer.

Melting range : Between 34° and 44°, determined by

Method II on a sample previously cooled to between 8° and 10°, Appendix 5.11.

Acid value : Not more than 1.0, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents, Appendix 3.3.15.

Iodine value : Between 18 and 36, determined on 0.80 g, Appendix 3.3.18.

Peroxide value : Not more than 20, Appendix 3.3.19.

Saponification value : Between 90 and 105, Appendix 3.3.20, boiling with the *0.5N alcoholic potassium hydroxide* for four hours.

Water absorption capacity : Weigh 10 g into a mortar. Add *water* in quantities of 0.2 to 0.5 ml from a burette and stir vigorously, incorporating all the *water* before adding the next quantity. The end-point is reached when visible droplets of water remain. Not less than 20 ml of *water* is absorbed.

Water-soluble acid or alkaline substances : Shake vigorously 5 g, previously melted on a water-bath, for two minutes with 75 ml of *water* previously heated to 90° to 95°. Cool to room temperature and filter through filter paper previously rinsed with *water*. To 60 ml of the filtrate, add 0.25 ml of *bromothymol blue solution*; not more than 0.2 ml of *0.02N hydrochloric acid* or 0.5 ml of *0.02N sodium hydroxide* is required to change the colour of the solution.

Water-soluble oxidisable substances : To 10 ml of the filtrate obtained in the test for **water-soluble acid or alkaline substances**, add 1 ml of *2N sulphuric acid* and *0.1N potassium permanganate*; the solution is not completely decolourised within ten minutes.

Ammonia : To 10 ml of the filtrate obtained in the test for **water-soluble acid or alkaline substances**, add 1 ml of *N sodium hydroxide* and boil; the vapours do not turn *red litmus paper* blue.

Chloride : Boil 1.0 g with 20 ml of *alcohol* under a reflux condenser, cool, add 1 ml of *2N nitric acid* and filter; to the filtrate add 5 drops of a 2.0 per cent w/v solution of *silver nitrate* in *alcohol*; any turbidity produced is not greater than that of a blank to which 0.5 ml of *0.02N hydrochloric acid* has been added.

Paraffins : Boil 0.5 g with 40 ml of *ethyl alcohol*; the solution is clear or not more than opalescent.

Sulphated ash : Not more than 0.15 per cent, Appendix 3.2.7, weigh accurately about 5 g, ignite, then add *sulphuric acid*.

Loss on drying : Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for one hour, Appendix 5.8.

Storage : Store in well-closed containers in a cool place.

Hydrous Wool Fat

Lanolin

Category : Pharmaceutical aid (water-in-oil emulsion ointment base).

Description : Yellowish-white, ointment-like mass; odour, slight and characteristic. On heating it separates at first into two layers; with continued heating with stirring, water is driven off and the residue which is transparent while warm, cools to form a yellowish, tenacious, soft mass.

Solubility : Practically insoluble in *water*; soluble in *chloroform* and in *solvent ether*, with the separation of water.

Standards : Hydrous Wool Fat is the purified, fat-like substance obtained from the wool of sheep.

Identification : Complies with the **Identification** tests described under Wool Fat.

Iodine value : Between 18 and 36, determined on about 0.8 g, accurately weighed, of the dried material, Appendix 3.3.18.

Peroxide value : Not more than 20, Appendix 3.3.19.

Water-soluble acid or alkaline substances : Warm 12.5 g with 150 ml of *water* on a water-bath, constantly stirring the mixture until the lanolin is melted; the fat separates completely on cooling, leaving the water layer nearly clear and neutral to *litmus solution*. Retain the water layer.

Water-soluble oxidisable substances : 10 ml of the filtrate obtained in the test for **Water-soluble acid or alkaline substances** comply with the test for **Water-soluble oxidisable substances** described under Wool Fat.

Ammonia : To 10 ml of the filtrate obtained in the test for **Water-soluble acid or alkaline substances**, add 1 ml of *N sodium hydroxide* and boil; the vapours do not turn *red litmus paper* blue.

Chloride : Complies with the test described under Wool Fat.

Paraffins : Boil 0.5 g of the dried lanolin obtained in the test for **Water**, with 40 ml of *ethyl alcohol*; the solution is clear or not more than opalescent.

Water : Between 25.0 per cent and 30.0 per cent w/w, determined by the following method: Weigh accurately about 3 g in a dish, heat on a water-bath until it separates into layers, and dry "in vacuo at 80°" over *silica gel*.

Storage : Store in tightly-closed, preferably rust-proof containers, in a cool place.

Dried Yeast

Category : Natural source of protein and Vitamin B Complex vitamins.

Dose : 2 to 10 g, four times a day.

Description : Buff or brownish-buff coloured flakes, granules or powder; odour, characteristic; taste, slightly bitter and characteristic.

Standards : Dried Yeast consists of unicellular fungi belonging to the family Saccharomycetaceae which have been dried by a process which avoids decomposition of the vitamins present. It contains not less than 45.0 per cent of protein, and in each g, the equivalent of not less than 0.3 mg of nicotinic acid, 0.04 mg of riboflavine and 0.1 mg of thiamine.

Identification : When examined under the microscope in water mounts, it shows somewhat angular masses of loosely agglutinated, rounded or ovoid cells, or short-branched filaments composed of united cells; cells colourless, about 3 to 8 µm in diameter and upto 12 µm in length; each with a somewhat granular protoplasm with one or two vacuoles and oil globules; cells occasionally containing four small spores. Very few starch grains may be present.

Microbial limits : The total microbial count by the *plate method*, Appendix 4.5, does not exceed 10,000 per g and the mould count does not exceed 100 per g; 1 g meets the requirements of the test for the absence of *Salmonellae*, and of *E. coli*.

Starch : To a suspension of 0.1 g in 10 ml of *water* add a few drops of *iodine solution*; no blue colour is produced.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Heavy metals : Not more than 10 parts per million, determined on 2.0 g by Method B, Appendix 3.2.4.

Ash : Not more than 8.5 per cent, Appendix 3.3.22.

Loss on drying : Not more than 9.0 per cent, determined on 0.5 g by drying in an oven at 100°, Appendix 5.8.

Assay : (a) *For Protein* – Weigh accurately about 1 g and carry out the *determination of nitrogen, Method A*, Appendix 3.3.5. Calculate the protein content by multiplying the nitrogen content so obtained by 6.25.

(b) *For Nicotinic acid* – Weigh accurately a quantity equivalent to about 500 µg of nicotinic acid. add 20 ml of *N sulphuric acid*, agitate vigorously, and wash down the sides of the flask with *N sulphuric acia*. Heat the mixture in an autoclave at 121° to 123° for thirty minutes, and cool. If lumping occurs, agitate the mixture until the particles are evenly dispersed. Adjust to pH 6.8 with *N sodium hydroxide*, transfer to a 100-ml volumetric flask, dilute to volume with *water* and mix to give the *test solution*. Filter before use if the solution is not clear. Carry out the *determination of nicotinic acid and nicotinamide*, Appendix 3.3.24.1, and express the result in mg of nicotinic acid per g.

(c) *For riboflavine* – On 2 g carry out the *determination of riboflavine*, Appendix 3.3.24.2, and express the result in mg of riboflavine per g.

(d) *For thiamine* – Carry out the *determination of thiamine*, Appendix 3.3.24.3, and express the result in mg of thiamine per g.

Storage : Store in tightly-closed containers.

Yellow Fever Vaccine

Category : Active immunising agent.

Dose : Prophylactic. By subcutaneous injection, not less than 1000 LD_{50} doses.

Standards : Yellow Fever Vaccine is an aqueous suspension chick embryo tissue infected with the 170 strain of yellow fever virus which is virulent for mice, but, although avirulent for man, has retained its immunising efficiency. The vaccine is prepared before use by reconstitution of the dried vaccine with Saline Solution or other appropriate solution so that the reconstituted vaccine is isotonic with blood.

The vaccine is produced on the basis of primary and seed lot systems. Secondary seed virus is injected into the embryos of fertile eggs which have been incubated for seven to eight days. After incubation for a further three to four days, the embryos are removed, pooled in batches, ground, and extracted with Purified Water. The suspension so obtained is centrifuged and the supernatant liquid to which a suitable stabiliser may be added, is then distributed into sterile glass ampoules and dried from the frozen state before the ampoules are sealed. The air is removed from the containers or replaced by *oxygen-free nitrogen* before the containers are sealed.

Description : White, slightly yellow or light brown pallets scales or powder.

The reconstituted vaccine complies with the following:

Identification : (A) When inoculated intracerebrally into susceptible mice, it produces encephalitis characteristic of infection by yellow fever virus, ending fatally within twenty-one days.

(B) When injected intracerebrally into healthy susceptible rhesus monkeys, it produces specific anti-

bodies, which can be demonstrated by appropriate virus neutralisation tests, in the blood of the majority of the monkeys injected. Symptoms of specific infection by yellow fever virus occurs infrequently.

Sterility : Complies with the *tests for sterility*, Appendix 4.6.

Undue toxicity : Inject ten times the human dose into each of two guinea-pigs weighing between 300 and 500 g; no symptoms of toxicity occur within twenty-one days.

Potency : Inject intracerebrally serial ten-fold dilutions into four to six week-old mice, both weighing between 11 and 14 g, using groups of not fewer than six mice for each dilution. Determine the LD_{50}, the quantity which causes the death of approximately one-half of the mice, within twenty-one days of injection, the animals exhibiting specific yellow fever virus encephalitis. The dose stated on the label contains not less than 1000 LD_{50}.

Storage : Store in the dark at a temperature of approximately 0°. When stored at higher temperature it loses its potency within a few days.

Labelling : The label on the container states (1) that the product is a living dried culture of 170 virus of yellow fever, prepared from infected chick embryo; (2) the name and volume of the liquid to be used for reconstituting the vaccine; (3) the dose; (4) the storage conditions; (5) the date after which it is not intended to be used.

Zinc Chloride

$ZnCl_2$ Mol. Wt. 136.29

Category : Pharmaceutical aid (for insulin preparations).

Description : White or practically white, crystalline powder or granules; odourless. Very deliquescent.

Solubility : Very soluble in *water*; freely soluble in *alcohol* and in *glycerin*.

Standards : Zinc Chloride contains not less than 95.0 per cent and not more than the equivalent of 100.5 per cent of $ZnCl_2$.

Identification : (A) To 2.0 g add 38 ml of freshly boiled and cooled *water* and add *2N hydrochloric acid* dropwise, until the solution is complete. The resulting solution gives the reactions of *zinc*, Appendix 3.1.

(B) A 5 per cent w/v solution in *2N nitric acid* gives the reactions of *chlorides*, Appendix 3.1.

pH : Between 4.6 and 6.0, determined in a solution prepared by dissolving 1.0 g in 9 ml of freshly boiled and cooled *water*, ignoring any slight turbidity, Appendix 5.10.

Ammonium salts : To 5 ml of a 10 per cent w/v solution add *N sodium hydroxide* until the precipitate first formed is redissolved, and then warm the solution; no odour of ammonia is perceptible.

Alkalis and alkaline earths : Not more than 1.0 per cent, determined by the following method: Dissolve 2.0 g in 150 ml of *water* in a 200-ml volumetric flask. Add sufficient *ammonium sulphide solution* to precipitate the zinc completely, dilute with *water* to volume, and mix. Filter through a dry filter, and reject the first few ml of the filtrate. To 100 ml of the subsequent filtrate add five drops of *sulphuric acid*, evaporate to dryness and ignite to constant weight.

Lead : Dissolve 0.50 g in 5 ml of *water* and transfer the solution to a *Nessler cylinder (A)*. Add 15 ml of *potassium cyanide solution Sp.*, mix and allow the mixture to become clear. In a similar, matched *Nessler cylinder (B)* place 5 ml of *water*, and add 2.5 ml of *standard lead solution* and 15 ml of *potassium cyanide solution Sp.* Add to each cylinder 0.1 ml of *sodium sulphide solution*. Mix the contents of each cylinder and allow to stand for five minutes. The solution in cylinder A is not more intensely coloured than the solution in cylinder B.

Sulphate : 2.0 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Oxychloride : Dissolve 1.5 g in 1.5 ml of freshly boiled and cooled *water*; the solution is clear or at most slightly opalescent. Add 7.5 ml of *alcohol*; the solution becomes cloudy but becomes clear again on the addition of 0.2 ml of *2N hydrochloric acid*.

Assay : Weigh accurately about 3 g, dissolve in about 125 ml of *water*, add 3 g of *ammonium chloride* and add sufficient *water* to produce 250.0 ml. To 25.0 ml of the resulting solution add 100 ml of *water*, 10 ml of *strong ammonia-ammonium chloride solution*, 1 ml of *eriochrome black T solution* and titrate with *0.05M disodium ethylenediaminetetraacetate* to a deep-blue end-point. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.006815 g of $ZnCl_2$.

Storage : Store in tightly-closed containers.

Zinc Oxide

ZnO Mol. Wt. 81.38

Category : Astringent; topical protectant.

Description : Soft, white or faintly yellowish-white powder, free from grittiness; odourless;

tasteles. It gradually absorbs carbon dioxide from air.

Solubility : Insoluble in *water*, and in *alcohol*; soluble in solutions of alkali hydroxides, and in dilute mineral acids.

Standards : Zinc Oxide contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of ZnO, calculated with reference to the ignited substance.

Identification : (A) It becomes yellow when strongly heated; the yellow colour disappears on cooling.

(B) A solution in *dilute hydrochloric acid* gives the reactions of *zinc*, Appendix 3.1.

Alkalinity : Mix 1 g with 10 ml of hot *water*, and add 2 drops of *phenolphthalein solution* if a red colour is produced, not more than 0.3 ml of *0.1N hydrochloric acid* is required to discharge it.

Carbonate and substances insoluble in acids : Mix 2 g with 10 ml of *water*, add 30 ml of *dilute sulphuric acid*, and heat on a water-bath, with constant stirring; no effervescence occurs and the resulting solution is clear and colourless.

Arsenic : Not more than 10 parts per million, Appendix 3.2.1.

Iron : Dissolve 0.1 g in a mixture of 5 ml of *water* and 0.5 ml of *iron-free hydrochloric acid* and dilute to 40 ml with *water*; the solution complies with *the limit test for iron*, Appendix 3.2.5, using 6 drops of *thioglycollic acid*.

Lead : Dissolve 2 g in a mixture of 20 ml of *water* and 5 ml of *glacial acetic acid* and add 5 drops of *potassium chromate solution*; the solution remains clear.

Loss on ignition : Not more than 1.0 per cent, determined on 2.0 g by igniting to constant weight at 500°.

Assay : Weigh accurately about 1.5 g and dissolve with 2.5 g of *ammonium chloride* in 50.0 ml of *N sulphuric acid* with the aid of gentle heat, if necessary. When solution is complete, add a few drops of *methyl orange solution* and titrate the excess sulphuric acid with *N sodium hydroxide*. Each ml of *N sulphuric acid* is equivalent to 0.04069 g of ZnO.

Storage : Store in well-closed containers.

Zinc Stearate

Category : Dusting powder.

Description : Fine, white, bulky powder, free from grittiness; odour, faint and characteristic.

Solubility : Practically insoluble in *water*, in *alcohol* and in *solvent ether*.

Standards : Zinc Stearate is a compound of zinc with a mixture of solid organic acids obtained from fats, and consists chiefly of variable proportions of zinc stearate and zinc palmitate. It contains the equivalent of not less than 12.5 per cent and not more than 14.5 per cent of ZnO.

Identification : (A) Add 1 g to a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid* and boil; an oily layer of fatty acids is produced on the surface of the liquid and the lower aqueous layer, after neutralisation, gives the reactions of *zinc*, Appendix 3.1.

(B) Separate the oily layer in the above test on a filter paper wetted with *water* and wash with boiling *water* until free from sulphate. Collect the fatty acid in a small beaker, allow to cool, pour off the supernatant water, melt the acid; filter while hot in a dry beaker, and dry at 105°, for twenty minutes; the melted acid congeals at a temperature not below 54°, Appendix 5.5.

Acidity or Alkalinity : A suspension is neutral to moistened *litmus paper*.

Alkalis and alkaline earths : Add 1 g to a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid*, boil, filter immediately and wash with 25 ml of hot *water*. Add *dilute ammonia solution* to make the filtrate just alkaline and then add *ammonium sulphide solution* in excess to precipitate the zinc as zinc hydroxide completely. Filter, add 0.5 ml of *sulphuric acid* to the filtrate, evaporate to dryness, and ignite the residue to constant weight; the residue weighs not more than 20 mg.

Free fatty acids : Mix 5 g with 100 ml of *solvent ether*, shake for half an hour, filter and evaporate 50 ml of the filtrate to dryness; the residue weighs not more than 50 mg.

Arsenic : Not more than 2 parts per million, Appendix 3.2.1.

Lead : Not more than 10 parts per million, determined in the following manner: Ignite 0.5 g in a platinum crucible for fifteen minutes in a muffle furnace at about 500°. Cool, add three drops of *nitric acid*, evaporate over a low flame to dryness, and ignite again at 500° for thirty minutes. Cool, dissolve the residue in 1 ml of a mixture of equal parts by volume of *nitric acid* and *water* and proceed as directed in the test for **Lead** in Magnesium Stearate beginning at the words "and wash into a separator....".

Assay : Weigh accurately about 1 g and boil with 50 ml of *0.1N sulphuric acid* until the fatty acid layer which separates is clear, adding more *water* as necessary to maintain the original volume, cool, and filter. Wash thoroughly with *water* until the last washing is not acid to *litmus paper*. Add to the combined filtrate and washings 15 ml of strong *ammonia-ammonium chloride solution* and 0.2 ml of *eriochrome black T solution*, heat the

solution to about 40° and titrate with *0.05M disodium ethylenediaminetetraacetate* until the solution is deep blue in colour. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.004069 g of ZnO.

Storage : Store in well-closed containers.

Zinc Sulphate

$ZnSO_4,7H_2O$ Mol. Wt. 287.54

Category : Astringent.

Description : Colourless, transparent crystals, or crystalline powder; odourless; taste, astringent and metallic. It is efflorescent in dry air.

Solubility : Very soluble in *water*; freely soluble in *glycerin*; insoluble in *alcohol*.

Standards : Zinc Sulphate contains not less than 99.0 per cent and not more than the equivalent of 108.0 per cent of $ZnSO_4,7H_2O$.

Identification : A solution (1 in 20) gives the reactions of *zinc*, and of *sulphates*, Appendix 3.1.

Clarity and colour of solution : A 5.0 per cent w/v solution is clear and colourless.

pH : Between 4.4 and 5.6, determined in a 5.0 per cent w/v solution, Appendix 5.10.

Aluminium, copper, magnesium, manganese and nickel : Dissolve 1 g in 20 ml of *water*, add *dilute ammonia solution* in excess, and allow to stand. The solution remains colourless, and no precipitate is produced within thirty minutes.

Alkalis and alkaline earths : Dissolve 2 g in about 150 ml of *water* contained in a 200-ml volumetric flask; precipitate the zinc completely by means of *ammonium sulphide solution*, and add *water* to make 200 ml, mix well, and filter through a dry filter rejecting the first portion of the filtrate. To 100 ml of the subsequent filtrate add a few drops of *sulphuric acid*; evaporate to dryness in a tared dish and ignite; the weight of the residue dose not exceed 5 mg.

Arsenic : Not more than 10 part per million, Appendix 3.2.1.

Iron : 0.14 g complies with the *limit test for iron*, using 6 drops of *thioglycollic acid*, Appendix 3.2.5.

Heavy metals : Not more than 10 parts per million, determined in the following manner: Dissolve 0.5 g in 5 ml of *water* and transfer the solution to a *Nessler cylinder (A)*. Add 10 ml of *potassium cyanide solution* mix and allow the mixture to become clear. Into a similar matched *Nessler cylinder (B)* add 5 ml of *water*, 0.5 ml of *standard lead solution* and 10 ml of *potassium cyanide solution*. Add to each cylinder 0.1 ml of *sodium sulphide solution*. Mix the contents of each cylinder and allow to stand for five minutes. The solution in cylinder A is not darker than that in cylinder B.

Chloride : 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

Assay : Weigh accurately about 0.3 g and dissolve in 100 ml of *water*. Add 5 ml of *strong ammonia-ammonium chloride solution*, 0.1 ml of *eriochrome black T solution* and titrate with *0.05M disodium ethylenediaminetetraacetate* until the solution is deep blue in colour. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.01438 g of $ZnSO_4,7H_2O$.

Storage : Store in tightly-closed containers.

Zinc Undecylenate

Zinc Undecenoate

$[CH_2:CH.(CH_2)_8,CO_2]_2Zn$ Mol. Wt. 431.92

Category : Antifungal (topical).

Description : Fine, white or pale yellowish-white powder.

Solubility : Practically insoluble in *water* and in *alcohol*.

Standards : Zinc Undecylenate is zinc di-(undec-10-enoate). It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{22}H_{38}O_4Zn$, calculated with reference to the dried substance.

Identification : (A) Acidify 5 g with 25 ml of *dilute sulphuric acid*, add 20 ml of *water* and extract in a separator with two quantities, each of 25 ml of *solvent ether*. Evaporate the ether extract until the odour of the solvent is not perceptible. Add *potassium permanganate solution* to 1 ml of the residue; the colour of the permanganate is discharged.

(B) To 3 ml of the residue obtained in **Identification** test (A) add 3 ml of freshly distilled *aniline* and heat for ten minutes in a test-tube at a rate such that the ring of condensate remains just below the mouth of the test-tube. Cool, add 10 ml of *alcohol* and 10 ml of *solvent ether*, wash the mixture with four quantities, each of 20 ml, of *water* and reject the washings. Evaporate the ether, add 5 mg of *decolorising charcoal*, filter, and evaporate the filtrate to dryness; the residue, after recrystallisation from *alcohol (70 per cent)*, melts at about 66°, Appendix 5.11.

(C) Ignite 0.1 g, cool, and dissolve the residue in *dilute hydrochloric acid*; the solution gives the reactions of *zinc*, Appendix 3.1.

Melting range : Between 115° and 121°, Appendix 5.11.

Acidity or Alkalinity : Suspend 2 g in 10 ml of *alcohol* and add 30 ml of *water*. Shake and add five drops of *phenol red solution*; not more than 0.2 ml of *0.1N hydrochloric acid* or *0.1N sodium hydroxide* is required for neutralisation.

Alkalis and alkaline earths : Carry out the test described under Zinc Stearate. The residue weighs not more than 20 mg.

Sulphate : 0.1 g, warmed with 25 ml of *water* containing 2.5 ml of *dilute hydrochloric acid* and filtered, complies with the *limit test for sulphates*, Appendix 3.2.8.

Loss on drying : Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Assay : Weigh accurately about 0.5 g and carry out the **Assay** described under Zinc Stearate, beginning at the words "boil with 50 ml of *0.1N sulphuric acid*.....". Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.02160 g of $C_{22}H_{38}O_4Zn$.

Storage : Store in well-closed containers.

Zinc Undecylenate Ointment

Zinc Undecenoate Ointment

Category : Antifungal (topical).

Standards : Zinc Undecylenate Ointment contains not less than 18.0 per cent and not more than 22.0 per cent of zinc undecylenate ($C_{22}H_{38}O_4Zn$), and not less than 4.5 per cent and not more than 5.5 per cent of free undecylenic acid ($C_{11}H_{20}O_2$), in a suitable ointment base.

Assay : (a) *For zinc undecylenate*—Weigh accurately about 2 g, add 20 ml of *dilute hydrochloric acid* and boil under a reflux condenser for at least twenty minutes or until the fatty layer is clear. Filter while hot and wash the residue with hot *water*. Cool the combined filtrate and washings, neutralise to *litmus paper* with *dilute ammonia solution*, add 3 ml of *dilute hydrochloric acid* and 5 g of *hexamine*, and titrate with *0.05M disodium ethylenediaminetetraacetate* using 0.4 ml of *xylenol orange solution* as indicator, until the colour becomes yellow. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.02160 g of $C_{22}H_{38}O_4Zn$.

(b) *For undecylenic acid*—Weigh accurately about 5 g, add 100 ml of *dilute hydrochloric acid* and heat to 70° with constant stirring. Cool and transfer to a separator with the aid of four quantities, each of 25 ml of *solvent ether* and add the rinsings to the mixture in the separator. Dilute the aqueous phase to 300 ml, saturate it with *sodium chloride* and shake the mixture. Transfer the aqueous layer to a second separator and extract with another 100-ml quantity of *solvent ether*. Wash the combined ether extracts with 10-ml quantities of *water* until the washings are free from chloride. Transfer the ether solution to a beaker and evaporate on a water-bath to about 5 ml. Add 20 ml of *carbon tetrachloride*, mix, transfer the mixture to a small separator and drain the carbon tetrachloride layer into a 100-ml graduated flask. Rinse the beaker with three quantities, each of 5 ml, of *carbon tetrachloride* and transfer the rinsings to the graduated flask, dilute to volume with *carbon tetrachloride* and mix. Evaporate 50.0 ml of the resulting solution to about 5 ml, add 100 ml of *alcohol*, previously neutralised, 3 drops of *phenolphthalein solution* and titrate the total undecylenic acid with *0.1N sodium hydroxide*. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01843 g of $C_{11}H_{20}O_2$. Calculate the content of free undecylenic acid from the difference between the total undecylenic acid and the undecylenic acid equivalent to the determined zinc undecylenate (the content of zinc undecylanate multiplied by 0.8533 gives the equivalent of undecylenic acid).

Storage : Store in tightly-closed containers. Avoid prolonged exposure to temperatures above 30°.

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Appendices

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APPENDIX 1

1. Apparatus for Tests and Assays

1.1 NESSLER CYLINDERS

Nessler cylinders which are used for comparative tests are matched tubes of clear colourless glass with a uniform internal diameter and flat, transparent base. They comply with Indian Standard 4161–1967. They are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50-ml mark 110 to 124 mm, the thickness of the wall 1.0 to 1.5 mm and the thickness of the base 1.5 to 3.0 mm. The external height to the 50-ml mark of the cylinders used for a test must not vary by more than 1 mm.

1.2 SIEVES

Sieves for pharmacopoeial testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material. The wires should be of uniform circular cross-section and should not be coated or plated. There must be no reaction between the material of the sieve and the substance being sifted.

Sieves conform to the following specifications:

1.3 THERMOMETERS

Unless otherwise specified, thermometers suitable for pharmacopoeial tests conform to Indian Standard 4825–1968 and are standardised in accordance with the 'Indian Standard Method of Calibrating Liquid-in-Glass Thermometers, 6274–1971.

The thermometers are of the mercury-in-glass type and are filled with a dried inert gas, preferably nitrogen. They may be standardised for total immersion or for partial immersion. Each thermometer should be employed according to the condition of immersion under which it was standardised. In the selection of the thermometer it is essential to consider the conditions under which it is to be used.

1.4 ULTRA-VIOLET LAMPS

The viewing of thin-layer chromatograms is done with the aid of a source of ultra-violet light such as a mercury vapour in quartz lamp. A suitable filter may be fitted to eliminate the visible part of the spectrum emitted by the lamp. Where the monograph prescribes the use of ultra-

Approximate sieve number*	Approximate percentage sieving area	Nominal mesh aperture size mm	Tolerance average aperture size ± mm	Approximate sieve number*	Approximate percentage sieving area	Nominal mesh aperture size mm	Tolerance average aperture size ± mm
4	55	4.0	0.13	44	38	355	13
6	51	2.8	0.09	60	37	250	13 (9.9)**
8	48	2.0	0.07	85	35	180	11 (7.6)
10	46	1.7	0.06	100	36	150	9.4 (6.6)
12	44	1.4	0.05	120	34	125	8.1 (5.8)
16	41	1.0	0.03	150	36	106	7.4 (5.2)
–	–	µm	± µm	170	35	90	6.6 (4.6)
22	37	710	25	200	36	75	6.1 (4.1)
25	36	600	21	240	34	63	5.3 (3.7)
30	38	500	18	300	35	53	4.8 (3.4)
36	36	425	15	350	34	45	4.8 (3.1)

*Sieve number is the number of meshes in a length of 2.54 cm in each transverse direction parallel to the wires.

**Figures in brackets refer to close tolerances; those without brackets relate to full tolerances.

violet light of wavelength 254 nm or 366 nm, an instrument consisting of a mercury vapour lamp and a filter which gives an emission band with maximum intensity at about 254 nm or 366 nm is used. The lamp should be capable of revealing without doubt a standard spot of sodium salicylate with a diameter of about 5 mm on a chromatographic plate coated with silica gel G. For this purpose carry out the following test:

Apply to a plate coated with *silica gel G*, 5µl of a 0.04 per cent w/v solution of *sodium salicylate* in *alcohol* for lamps of maximum output at 254 nm and 5 µl of a 0.2 per cent w/v solution in *alcohol* for lamps of maximum output at 366 nm. Examine the spot in a position normal to the radiation. The distance between the lamp and the plate under examination used in a pharmacopoeial test should not exceed the distance used to carry out the above test.

1.5 VOLUMETRIC GLASSWARE

Volumetric apparatus is normally calibrated at 27°. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25°. This discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27°.

Pharmacopoeial assays involving volumetric measurements require the use of accurately calibrated glassware. Volumetric apparatus must be suitably designed to assure accuracy. The design, construction and capacity of volumetric glassware should be in accordance with those laid down by the Indian Standards Institution. The tolerances on capacity for volumetric flasks, pipettes and burettes, as laid down in the relevant Indian Standards, are set out in the following table.

1.6 WEIGHTS AND BALANCES

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to be "accurately weighed", the weighing is to be performed so as to limit the error to not more than 0.1 per cent. For example, a quantity of 50 mg is to be weighed to be nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and a quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

Volumetric Flask: I.S. 915–1975

Nominal capacity, ml	5	10	25	50	100	250	500	1000
Tolerance, ± ml	0.02	0.02	0.03	0.04	0.06	0.1	0.15	0.2

One Mark Pipettes: I.S. 1117–1975

Nominal capacity, ml	1	2	5	10	20	25	50	100
Tolerance, ± ml	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.06

Graduated Pipettes: I.S. 4162–1967

Nominal capacity, ml	1	2	5	10	25
Subdivision, ml	0.01	0.02	0.05	0.10	0.2
Tolerance, ± ml	0.006	0.01	0.03	0.05	0.1

Burettes: I.S. 1997–1967

Nominal capacity, ml	10	25	50	10
Subdivision, ml	0.05	0.05	0.1	0.1
Tolerance, ± ml	0.01	0.03	0.05	0.1

2. Biological Tests and Assays

2.1 DESIGN AND ANALYSIS OF BIOLOGICAL ASSAYS

1. Introduction

Biological assays (bioassays) are prescribed where the potency of a drug substance or its preparation cannot be adequately determined by chemical or physical means, but where it may be possible to observe the biological effect of the drug on some type of living matter. The principle of such assays is to compare how much of a sample being tested produces the same biological effect as a given quantity (the Unit) of a standard preparation. It is essential to ensure that the conditions, under which the sample and the standard preparation are tested, are identical in all respects of time, environmental factors and biological media used.

However, even with the best of efforts by the analyst to carry out the tests under comparable conditions, the estimates of potency may still be subject to random errors due to difficulties of perfectly matching and controlling the experimental conditions or the inherent variability of biological responses. Therefore, in biological assays, it is necessary not only to estimate the potency of a drug but also to compute the margin of errors in the estimate.

The typical bioassay involves a stimulus (e.g. a vitamin, a drug, a fungicide) applied to a subject (e.g. an animal, a tissue, or bacterial culture). The intensity of the stimulus applied to a subject is known as the dose, and is measured by a weight, a volume or a concentration of the preparation, or some mathematical transformation of the same (logarithm, square root, etc.). The observed effect of the stimulus on the subject, known as the response, may be measured by the total weight or the weight of some organ of the subject, the diameter of the inhibition zone for a bacterial colony, an analytical value such as blood sugar content, or the number of organisms in a unit volume, or even a simple record of occurrence or non-occurrence of some physiological symptoms.

Methods for the design of assays and the calculation of their errors are described below. Alternative assay designs or methods of calculation may be used provided that they are not less reliable than those described here.

2. Precision of Biological Assays

On the evidence of biological assays, it is not possible to make a precise statement of the actual limits within which the potency of a preparation is certain to lie. It is the usual convention that if there is a 95% probability that the true potency will be within the limits specified, then this is deemed equivalent to certainty. Occasionally, a 99% probability level is used.

The limits are derived from the estimated errors due to random variations in experimental results. However, even the estimation of the error itself may be subject to error, except when there are a very large number of observations. Allowance has to be made for this in setting up the *fiducial (or confidence) limits*, that is, limits within which we can say that the true potency will be in 95% or 99% of the experiments.

It is to be noted that the error may be estimated in two ways: (a) from an *internal estimate* from the actual assay itself or (b) a *direct estimate* from several similar experiments, that is, experiments conducted under identical conditions for all factors within control. From the methods prescribed in the pharmacopoeia, it will be seen that internal estimates are not always possible. Even if it is possible, it will have a value only if the experiments with the standard as well as the test preparation are conducted under strictly comparable conditions in all other respects. Direct estimates on the other hand take into account any variation from time to time or from experiment to experiment, and therefore include all sources of variation. However, direct estimates will be reliable only if a sufficiently large number of assays conducted in identical form or design are available. Moreover, any such direct estimates given in the Pharmacopoeia may not apply to a similar experiment done in a particular laboratory, unless the sources of variation are the same as occurring in the given estimates.

Potency estimates are generally made on the basis of the logarithms of the doses (*see* Section 4). Since potency is the ratio of the doses required to produce the same response, the difference between the logarithms of these doses gives the logarithm of the potency. The errors of the estimates are also calculated on the logarithms of the doses. The actual potency can be obtained from log potency by taking its antilogarithm, and similarly from the fiducial limits of the log potency, the actual fiducial limits can be obtained. However, as an approximation, the error can be converted into a percentage (provided this is less than 10 per cent) and the fiducial limits can be calculated from this percentage error. The error as well as the fiducial limits can be given in actual values or expressed as percentages of the potency estimate.

Where the terms 'stated' potency and 'estimated' potency are used in this section and elsewhere, the following definitions are intended:

Stated potency — This is a nominal value assigned to a formulation or preparation from knowledge of the

potency of the bulk material or, in the case of bulk drugs, it may be a calculated potency.

Estimated potency—This is the potency calculated from the data obtained from assays.

3. Estimation of Error from Sets of Similar Observations

An estimate of the error can be derived from a statistical measure called the Variance. Consider an experiment in which an independent observations have been made of a quantity X. Then the average or mean of these n observations. denoted by $\bar{X}$, can be calculated as:

$$\bar{X} = \frac{\Sigma X}{n} \qquad \ldots (1)$$

where X denotes the individual observations and Σ denotes that the n observations have to be summed up. Let $d = X - \bar{X}$ be the deviation of each observation from the mean. Some of the d values will be positive and others negative and it can be shown that the sum of all the n deviations will total upto zero. It is usual statistical practice to take the squares of the d values (d^2) to eliminate the positive and negative values and define the Variance as the mean of the sum of squared deviations.

In other words

$$V = s^2 = \frac{\Sigma d^2}{n-1} \qquad \ldots (2)$$

where V is the Variance; s is called the standard deviation.

A denominator of $(n-1)$ called the degrees of freedom is used rather than n, the total number of observations, since out of the n deviations, if $(n-1)$ are independently known, the nth deviation as automatically determined to make $\Sigma d = 0$.

If we have several sets of n separate observations and for each set the Variance, assumed to be the same, has been calculated, then a pooled estimate of the common Variance may be calculated by formula

$$V = s^2 = \frac{\Sigma (\Sigma d^2)}{\Sigma (n-1)} \qquad \ldots (3)$$

The above formula may be used even if the mean values of each set may differ, but if there is evidence that the Variances in each set are substantially different, then equation (3) should not be used for a pooled Variance.

The computations of the Variance by formula (2) may be tedious and subject to errors of rounding off if the mean X is not a round figure. An alternative quick method for calculating V is

$$V = \frac{\Sigma X^2 - nX^2}{(n-1)} \qquad \ldots (4)$$

It should be noted that in the above formula each deviation d need not be computed.

The standard deviation s $(=\sqrt{V})$ gives the "error" of a single observation from the expected value.

The error attached to the mean $\bar{X}$ of a set of n observations, called the Standard Error (S.E.) of the mean is given by

$$S_m = \sqrt{V/n} = \frac{s}{\sqrt{n}} \qquad \ldots (5)$$

Let us define a quantity $t = \frac{\bar{X}}{S_m} = \frac{\bar{X}}{s/\sqrt{n}}$

then from statistical methods the distribution of the t values can be derived.

Further, given a level of probability such as P= 0.95 or P= 0.99, the value of t for given degrees of freedom can be computed. The interpretation is that, for a given P level the observed value of t will be less than the tabulated value with probability P. The observed value will exceed the tabulated value with probability $1 - P$.

These concepts enable to set fiducial limits of error in the estimated potency of a test preparation calculated by the formula

$$\bar{X} \pm {}'0.95\, s/\sqrt{n} \qquad \text{for 95 per cent level} \qquad \ldots (6)$$

$$\bar{X} \pm {}'0.99\, s/\sqrt{n} \qquad \text{for 99 per cent level}$$

where '0.95 or '0.99 denotes the tabulated t value at 95 per cent or 99 per cent points.

A condensed table of t values for 95 per cent and 99 per cent levels is given in Table 1.

Extensive tables may be found in standard statistical tables.

4. Direct Estimates of Error from Repeated Assays

Methods given in Section 3 may be applied to calculate the direct estimate of error for an assay from observed results of several similar assays or sets of similar assays. The observations may be of the actual potency or the logarithm of the potency. Log potency may be used only if the standard deviation is less than 10 per cent of a single assay.

These calculations are shown in the following examples:
Example 1 applies to the case of several similar assays and Example 2 to the case of a set of assays.

TABLE 1 – 95 and 99 Percentage Points of 't'

Degrees of Freedom	95 per cent Points	99 per cent Points
1	12.706	63.657
2	4.303	9.925
3	3.182	5.841
4	2.776	4.604
5	2.571	4.032
6	2.447	3.707
7	2.365	3.499
8	2.306	3.355
9	2.262	3.250
10	2.229	3.169
11	2.201	3.106
12	2.179	3.055
13	2.160	3.012
14	2.145	2.977
15	2.131	2.947
16	2.120	2.921
17	2.110	2.898
18	2.101	2.878
19	2.093	2.861
20	2.086	2.845
21	2.080	2.831
22	2.074	2.819
23	2.069	2.807
24	2.064	2.797
25	2.060	2.787
30	2.042	2.750
40	2.021	2.704
50	2.008	2.678
60	2.000	2.660
80	1.960	2.576

Example 1 : Several Similar Assays

No.	Estimate of potency from individual assays X	Percentage over mean $(X/\bar{X}) \times 100$ Y
1	1.069	102.24
2	1.131	108.17
3	0.994	95.07
4	1.051	100.52
5	1.087	103.96
6	1.000	95.64
7	0.987	94.40
Total	7.319	700.00

$\bar{X} = 7.319/7 = 1.04557$ $\bar{Y} = 700.00/7 = 100.0$

$\Sigma X^2 = 7.67030$ $\Sigma Y^2 = 70162.39$

$$V = \frac{7.67030 - 7 \times (1.04557)^2}{6} \qquad V = \frac{70162.39 - 7 \times 100^2}{6}$$

$= 0.0177836/6$ $= 162.39/6$

$= 0.002964$ $= 27.065$

$s = \sqrt{V} = 0.054442$ $s = 5.20$

$s/\bar{X} = 0.054442/1.04557$ $s/\bar{Y} = 5.20/100$

$= 5.20\%$ $= 5.20\%$

For P = 0.95 and 6 degrees of freedom t = 2.447 (from Table 1)

Fiducial limits of error

$= 100 \pm (2.447)\ (5.20)$

$= 100 \pm 12.7$

$= 87.3$ to 112.7 per cent

These limits of error apply to any single estimate of potency from an individual assay of the type considered.

Example 2 : Several Sets of Assays

(Error limits are computed for log potency)

No.	Preparation 1		Preparation 2	
	Potency each assay	Log potency	Potency each assay	Log potency
1	1.069	0.0290	0.976	− 0.0106
2	1.131	0.0535	0.808	− 0.0926
3	0.994	− 0.0026	0.719	− 0.0367
4	1.051	0.0216	0.877	− 0.0570
5	1.087	0.0362		
6	1.000	0.0000		
7	0.987	− 0.0057		
Total		0.1320		− 0.1969
Mean		0.01886		− 0.04923

$s_1^2 = 0.003030/6$ $\qquad$ $s_2^2 = 0.003591/3$

Pooled Variance s^2

$$= \frac{0.003030 + 0.003591}{6 + 3}$$

$$= 0.006621/9 = 0.0007357$$

$$s = 0.02712$$

For P = 0.95 and 9 degrees of freedom, t = 2.262

Log fiducial limits of error (per cent)

$$= 2 \pm (2.262)\ (0.02712)$$

$$= 2 \pm 0.06135$$

$$= 1.9387 \text{ to } 2.0614$$

Fiducial limits of error : 86.8 to 115.2 per cent

These limits of error apply to any single estimate of potency from an individual assay.

5. Assays Depending on Individual Effective Dose

In an assay where the effective dose (lethal dose, etc.) is obtained for each subject (animal or tissue or culture) separately, using a set of n_s subjects for the standard and a set of n_t subjects for the test preparation, let $\bar{X}_s$ and $\bar{X}_t$ be the mean log effective doses for the standard and test preparations. Then the log potency $M = \bar{X}_s - \bar{X}_t$.

It is assumed that the same or equivalent concentrations are used in both the cases. If any deliberate dilution factor has been used, the final potency estimates and the fiducial limits must be multiplied by the appropriate factor.

The Variance of M is the sum of the Variances of the two means and is calculated by the formula

$$S_M^2 = S_x^2 \left(\frac{1}{n_s} + \frac{1}{n_t} \right) \qquad \ldots(7)$$

where

$$S_x^2 = \frac{(\Sigma X_s^2 - n_s \bar{X}_s^2) + (\Sigma X_t^2 - n_t \bar{X}_t^2)}{n_s + n_t - 2} \qquad \ldots(8)$$

The fiducial limits are :

$$\text{antilog } (M \pm t\, S_M) \qquad \ldots(9)$$

Example 3 : Individual Lethal Dose

Standard	Estimate of individual lethal dose ml/kg	Log	Test preparation	Estimate of individual lethal dose ml/kg	Log
	1.23	0.0899		1.13	0.0531
	1.33	0.1239		1.16	0.0645
	1.03	0.0128		1.07	0.0294
	1.18	0.0719		1.03	0.0128
	1.30	0.1139		1.26	0.1004
	1.31	0.1173			
	1.21	0.0828			
	1.13	0.0531			
Total		0.6656	Total		0.2602

$n_s = 8, n_t = 5$

$\bar{X}_s = 0.0832, \bar{X}_t = 0.05204$

$$S_x^2 = \frac{0.009797 + 0.004548}{8 + 5 - 2} = 0.001304$$

$M = 0.0832 - 0.05204 = 0.03116$

Potency ratio = antilog M = 1.0744

$$S_M^2 = 0.001304 \frac{1}{8} + \frac{1}{5} = 0.0004238$$

$S_M = 0.02059$

For P = 0.95 and 11 degrees of freedom t = 2.201

Log fiducial limit of error

$= 0.03116 \pm (2.201)\ (0.02059)$

$= 0.03116 \pm 0.04532$

$= -0.01416$ to 0.07648

$= 1.9858$ to 0.0765

Actual fiducial limits

$= 0.9679$ to 1.192

6. Assays Depending upon Measured Effects on Individual Biological Systems

In this method of assay the effects of drugs on biological systems are measured, e.g. whole animal, isolated animal tissue or bacterial culture. The responses may be in the nature of weight gains, organ weights, size of zones of inhibition of bacterial growth, etc.

The methods of computation described in this section will be valid only if the following conditions are fulfilled :

(a) The responses to each treatment follow a statistical normal distribution.

(b) The standard deviation of the response is independent of the level of response.

(c) Within the range of doses used the relationship between the logarithm of the dose and the response can be represented by a straight line.

(d) The straight line for any test preparation must be parallel to the line determined by the standard.

(e) The allocation of the experimental units to the different treatment groups has been made on a random basis.

When conditions (c) and (d) do not hold, it may still be possible to overcome the difficulty by using some other transformed form of the response such as square or logarithm of the response.

If any of the above conditions do not hold, then the methods given in this section will not be reliable and a special statistical study of the situation will be necessary.

To simplify the statistical computations the following further additional restrictions will have to be imposed on the assay design:

(i) Each preparation of the assay must have the same number of dilutions.

(ii) The ratio between adjacent doses must be constant for all treatments in the assay.

(iii) There are an equal number of responses to each treatment.

(Sometimes one response or one treatment group may be missing. Such cases will require more complex statistical analysis).

6.1 Assay design : The allocation of the individuals to the different treatments may be made in various ways.

Random design – Where experimental units appear to be reasonably homogenous. then a randomly selected unit may be assigned to different treatments. The random selection can be made using Random Number Tables.

Randomised block – Where it is possible to segregate an identifiable source of variation, then the experimental units are divided into different blocks so that variation of the units within blocks will be substantially smaller than overall variation between blocks. Such homogenous groups (blocks) must be formed before the experiment starts. All the treatments must be tried on experimental units in each block and the choice of an individual within the block to a treatment must be made through a random process.

There are many other types of designs such as Latin squares, partially balanced block, Cross-over test, etc. Reference may be made to specialised statistical texts on experimental design for further details.

6.2 Analysis of variance (Anova) : In almost all designed experiments or assays, the basic statistical analysis of data is similar and uses methods of Analysis of variance. These methods will be presented here assuming that in the various designs, the standard and test preparations are

tested at two or three dosage levels (S_1, S_2, S_3 and T_1, T_2, T_3).

The linear contrasts, relating the slope of the log-response lines can be derived when there are at least two dose levels. With three dose levels the quadratic contrasts will represent the curvature of the lines.

The formulae for the assays are tabulated below:

TABLE 2 – Formula for Assays with Two Doses of each Preparation

	Standard (S)	Test Preparation (T)
Low dose (total response)	S_1	T_1
High dose (total response)	S_2	T_2
For preparation (total response)	$S_1 + S_2 = S$	$T_1 + T_2 = T$
Linear contrast	$S_2 - S_1 = L_s$	$T_2 - T_1 = T_s$

TABLE 3 – Formula for Assays with Three-dose Levels of each Preparation

	Standard (S)	Test Preparation (T)
Low dose	S_1	T_1
Middle dose	S_2	T_2
High dose	S_3	T_3
For preparation	$S_1 + S_2 + S_3 = S$	$T_1 + T_2 + T_3 = T$
Linear contrast	$S_3 - S_1 = L_s$	$T_3 - T_1 = L_t$
Quadratic contrast	$S_1 - 2S_2 + S_3 = Q_s$	$T_1 - 2T_2 + T_3 = Q_t$

Suppose there are N experimental units in the experiment. Then the total variation in the responses is first of all computed over the N units. Then by different combinations of the results of the various experimental units, the total variation in responses is partitioned into different segments specific to a source of variation as shown in Table 4. Let K denote the square of the sum of all the responses divided by N, the total number of responses in the experiment.

$$K = (\Sigma y)^2/N$$

TABLE 4 – Tests of Validity

Source of Variation	Degrees of Freedom	Reduced Sum of Squares: Two-dose assay	Reduced Sum of Squares: Three-dose assay
Preparation	1	$\frac{S^2 + T^2}{2n} = K$	$\frac{S^2 + T^2}{3n} = K$
Regression	1	$\frac{(L_s + L_t)}{2n} = E$	$\frac{(L_s + L_t)}{2n} = E$
Parallelism	1	$\frac{(L_s^2 + L_t^2)}{2n} - E$	$\frac{(L_s^2 + L_t^2)}{2n} - E$
Quadratic	1	–	$\frac{(Q_s + Q_t)^2}{12n} = Q$
Difference of quadratics	1	–	$\frac{Q_s^2 + Q_t^2}{6n} = Q$

The residual error of the assay is obtained by subtracting the variations allowed for in the design as per formula above from the total variations in response. To analyse the significance of the sources of variation in Table 4 each of the reduced sums of squares obtained from the table should be divided by the corresponding degrees of freedom to give mean squares. Similarly the mean square for the residual error (S^2) can be computed. Details are given in Table 5.

TABLE 5 – Estimation of Residual Error

Source of Variation	Degrees of Freedom	Reduced Sum of Squares: Random design	Reduced Sum of Squares: Randomized block
Treatments	k–1	$\frac{S_1^2 + S_2^2}{n} - K$	$\frac{S_1^2 + S_2^2}{n} - K$
Blocks (rows)	n–1	–	$\frac{R_1^2 + R_2^2}{k} - K$
Residual error by subtraction		•	•
Total	N–1	$\Sigma y^2 - K$	$\Sigma y^2 - K$

Note : y denotes the individual response.

TABLE 6 – The Variance Ration (F) Distribution

f_2 Degrees of freedom for denominator	f_1 Degrees of freedom for numerator									
	1	2	3	4	5	6	7	8	20	∞
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.54	2.30
	9.33	6.93	5.95	5.41	5.06	4.82	4.64	4.50	3.86	3.36
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.33	2.07
	8.68	6.36	5.42	4.89	4.56	4.32	4.14	4.00	3.37	2.87
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.12	1.84
	8.10	5.85	4.94	4.43	4.10	3.87	3.70	3.56	2.94	2.42
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	1.93	1.62
	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	2.55	2.01
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	1.75	1.39
	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.20	1.60
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.57	1.00
	6.63	4.61	3.78	3.32	3.02	2.80	2.64	2.51	1.88	1.00

The upper values correspond to P = 0.05, the lower values to P = 0.01

The ratio of the mean square for each source of variation to mean residual error defines a statistical quantity called F ratio (variance ratio). Values of F for given probability levels and the two degrees of freedom namely that of the numerator and denominator in the F ratio are given in Table 6.

If the observed value of a F ratio is larger than the critical value of F, for a given probability level, then the variable for which this test is done is said to be "significant" at the probability level. As long as the observed F value is less than the tabulated critical value we may deem that the observed variation may be attributable to random causes.

6.3 Estimation of potency and fiducial limits : Compute the mean response ($\bar{Y}_s$, $\bar{Y}_t$) for each preparation by formula :

$$\bar{Y}_s = \frac{S}{N_s} \; ; \; \bar{Y}_t = \frac{T}{N_t} \qquad \ldots(10)$$

Compute b, the slope for assays with two doses of each preparation by the relation

$$b = \frac{L_s + L_t}{In} \qquad \ldots(11)$$

where I is the interval between adjacent log doses of any preparation.

For assays with 3-dose levels the denominator in the above expression would be 4 In.

The logarithm of the potency ratio of a test preparation given by expression

$$M_t = \frac{-\bar{Y}_t - \bar{Y}_s}{b} \qquad \ldots(12)$$

is an estimate of the true potency of the unknown.

Fiducial limits may be computed as under:

$$C\,M_t \pm \sqrt{(C-1)\,(CM_t^2 + C'\,I^2)} \qquad \ldots(13)$$

where C′ is a constant (C = 1 for 2 x 2 dose, 1 test preparation; and C′ = 8/3 for 3 x 3 dose, 1 test preparation)

$$C = E/(E - s^2\,t^2) \qquad \ldots(14)$$

E is computed as per formula in Table 4.

s^2 is mean square error from Analysis of Variance (Table 5).

t is the value found from Table 1 according to the degrees of freedom for s^2.

C is a measure of the significance of regression and in an assay with a well-defined slope the value of C will be very close to unity.

The use of the above formulae is illustrated in Example 4, an assay where the standard and test preparations are compared at two dose levels, the number of tests being replicated (blocks) 4 times for each dose level.

Example 4: Two-dose Multiple Assay Randomised Block Design

Response Data

Block	Standard		Test		Block total
	S_1	S_2	T_1	T_2	
1	19.0	25.0	16.0	20.5	$R_1 = 80.5$
2	19.1	19.0	15.5	22.0	$R_2 = 75.6$
3	18.2	21.9	14.0	18.0	$R_3 = 72.1$
4	19.8	25.0	18.5	19.8	$R_4 = 83.1$
Mean	19.03	22.73	16.00	20.08	

S_1 = 0.2 ml of standard; T_1 = 0.2 ml of test
S_2 = 0.4 ml of standard; T_2 = 0.4 ml of test

Response Totals and Contrasts as per Table 2

	Standard	Test	Total
Low dose	$S_1 = 76.1$	$T_1 = 64.0$	
High dose	$S_2 = 90.9$	$T_2 = 80.3$	
Preparation totals	$S = 167.0$	$T = 144.3$	$\Sigma Y = 311.3$
Linear contrast	$L_s = 14.8$	$L_t = 16.3$	$\Sigma L = 31.1$

Analysis of Variance as per Table 5
(*see* computational explanation below)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F	P
Preparations	1	32.2057	32.2057		
Regression	1	60.4506	60.4506	20.31	0.01
Parallelism	1	0.1407	0.1407	0.05	NS
Treatments	3	92.7970	30.9323	10.39	0.01
Blocks	3	18.1769	6.0589	2.04	NS
Error	9	26.7855	2.9762		
Total	15	137.7594			

NS–Not significant

Number of treatments (observations) 16; hence, total degrees of freedom = 16 − 1 = 15

Number of observations in each block 4; hence, block degrees of freedom = 4 − 1 = 3

Number of doses in experiment 4; hence, treatment degrees of freedom = 4 − 1 = 3

n = Number of treatments per dose; d = Number of dose levels.

$$K = (\Sigma y)^2/N = (311.3)^2/16 = 6056.7306$$

$$\text{Preparation} = \frac{S^2 + T^2}{dn} - K$$

$$= 6088.9363 - 6056.7306$$

$$= 32.2057$$

$$\text{Regression} = (L_s + L_t)^2/4n = 967.21/16$$

$$= 60.4506 = E$$

$$\text{Parallelism} = (L_s^2 + L_t^2)/2n - E$$

$$= 484.73/8 - E$$

$$= 60.5913 - 60.4506$$

$$= 0.1407$$

$$\text{Blocks} = (R_1^2 + R_2^2 + R_3^2 + R_4^2)/4 - K$$

$$= 24299.63/4 - K$$

$$= 6074.9075 - 6056.7306$$

$$= 18.1769$$

$$\text{Total} = \Sigma y^2 - K$$

$$= 6194.49 - 6056.7306$$

$$= 137.7594$$

Validity of assay – The analysis of variance satisfactorily confirms that there is significant regression between dose levels and there is no departure from parallelism since mean square attributed to parallelism is not statistically significant. Look up Table 6 to find probability of observed ratio for given degrees of freedom.

Calculation of potency ratio and fiducial limits

$$E = 60.4056$$

$$I = \log 0.4 - \log 0.2 = 0.3010$$

Error square has 9 degrees of freedom $t = 2.262$ at 95 % P

$$S^2 = 2.9762$$

$$b = (L_s + L_t)/8I = 31.1/(8 \times 0.3010)$$

$$= 12.9153$$

$$\overline{Y}_t = 144.3/8 = 18.0375, \ \overline{Y}_s = 167.0/8$$

$$= 20.875$$

$$M_t = (\overline{Y}_t - \overline{Y}_s)/b$$

$$= (18.0375 - 20.875)/12.9153$$

$$= -2.8375/12.9153$$

$$= -0.2197$$

Potency ratio of test preparation :

$$\text{Antilog } M_t = 0.6030$$

$$C = 60.4056/[60.4056 - (2.262)^2 \times 2.9762]$$

$$= 60.4056/(60.4056 - 15.2282)$$

$$= 60.4056/45.1774 = 1.337$$

Log fiducial limits to the potency ratio :

$$1.337 \ (-0.2197)$$

$$\pm \sqrt{0.337[1.337 \times (-0.2197)^2 + 0.3010^2]}$$

$$= -0.2937 \pm \sqrt{0.05228}$$

$$= -0.2937 \pm 0.2286$$

$$= -0.5223 \text{ to } -0.0651$$

Fiducial limits to potency ratio

$$= \text{antilog } (-0.5223) \text{ to antilog } (-0.0651)$$

$$= 0.3004 \text{ to } 0.8608$$

7. Assays Depending upon Quantal Responses

In certain assays such as that for insulin by subcutaneous injection into mice, it is impossible or very difficult to measure the effect on each animal on a graduated quantitative scale. Instead, the effect such as death or hypoglycaemic symptoms may be observed as either occurring or not occurring in each animal and the result depends on the number of animals in which it occurs. Such assays are known as quantal or all-or-none.

These situations can also be analysed according to the methods given in Section 6. In place of n separate responses to each treatment, a single value is recorded such as the percentage of test bodies showing a positive quantal response. The relationship of such percentage when plotted against the logarithm of the doses will be in the form of an S-curve (sigmoid curve). Since one of the assumptions for validity of the methods given in Section 6 is the linear relation between response and log dose, the S-curve must somehow be transformed to get a linear form prior to using the methods.

The most common methods of transformations are change of percentages to probits, logits or angles.

Full details of methods should be obtained from standard statistical text books or pharmacology or biological assays. However, the following approximate methods may be adequate provided extremes of 0 per cent or 100 per cent quantal responses do not occur.

The percentage of test bodies (animals) giving a positive response to each treatment is converted to equivalent probits using Table 7.

These probits may then be taken as the response values for use in methods of Section 6. A weighting

TABLE 7 – Probits Corresponding to Percentages

	0	1	2	3	4	5	6	7	8	9
0	–	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

TABLE 8 – Weighting Coefficients Corresponding to Probits

Probits 1	2	3	4	
0.0	0.001	0.015	0.131	0.439	–
0.1	0.001	0.019	0.154	0.471	0.9
0.2	0.001	0.025	0.180	0.503	0.8
0.3	0.002	0.031	0.208	0.532	0.7
0.4	0.002	0.040	0.238	0.558	0.6
0.5	0.003	0.050	0.269	0.581	0.5
0.6	0.005	0.062	0.302	0.601	0.4
0.7	0.006	0.076	0.336	0.616	0.3
0.8	0.008	0.092	0.370	0.627	0.2
0.9	0.011	0.110	0.405	0.634	0.1
–	0.015	0.131	0.439	0.637	0.0
	8	7	6	5	
	Probits				

coefficient (w) corresponding to each probit is obtained from Table 8.

Formulae for sums of squares, analysis of variance are the same as given in Tables 4 and 5, except for the computation of the error term (s^2) This should be calculated using the formula:

$$s^2 = \frac{k}{n \Sigma w} \quad \ldots(15)$$

where k is the number of treatments, n is the number of animals tested in each treatment group and Σw is the sum of the weighting coefficients.

Potency and fiducial limits are calculated as before using equations (12) and (13).

8. Combination of Potency Estimates

When the same preparation is assayed several times, the resulting set of potencies can be combined into a single value to give an overall potency. A simple method is described here, but it should be noted that,

(a) estimates of log potency should be corrected by the assumed potency before they are combined, and

(b) each estimate should have been obtained from a separate assay which gave a set of responses to the test preparation as well as to the standard preparation.

It is assumed that individual potency estimates have been analysed to give the relevant numbers of values of log potencies (M) with associated fiducial limits in logarithms.

For each assay the logarithmic fiducial interval (L) is obtained by subtracting the lower limit from the upper one. A weight (W) for each value of M is obtained from the equation:

$$W = 4t^2/L^2 \quad (16)$$

where t is the value in Table 1 corresponding to the degrees of freedom for the error mean square in the appropriate analysis of variance

The logarithm of the weighted mean potency ($\overline{M}$) is obtained from the equation

$$\overline{M} = \Sigma WM/\Sigma W \qquad (17)$$

The standard error of the mean potency ($s_{\overline{M}}$) is taken to be the squareroot of the reciprocal of the total weight

$$s_M = \sqrt{1/\Sigma W} \qquad \ldots(18)$$

The fiducial limits with 95 per cent probability are obtained from the expression antilog $\overline{M} \pm ts_{\overline{M}}$. The appropriate value of t in Table 1 is that corresponding to the sum of the numbers of degree of freedom for the error mean square in the individual assays.

This approximate method should be satisfactory provided that

(a) the individual estimations are mutually consistent,

(b) C (equation 14) is less than 1.1 for each of the total number of assays.

NOTE — All the statistical methods given here are valid only if a random procedure is followed in the selection of the subjects (animals etc.), whether it is a simple random sample, or a batch by batch sample as in a randomised block design. It is clear that if there is any systematic difference between the samples of animals chosen for two different doses or preparations, the effect of differences observed will not be a measure of the actual differences.

In taking a random sample of subjects, we assume that it has been chosen from a very large group. But in practice, this is strictly not true. The number of subjects from which the experimenter has to choose his samples will be usually limited. He has then to assume that this limited number available at any time is a strictly random sample from the hypothetical population in which he is interested. For comparative experiments, as in the investigation of relative toxicity, or relative potency of a drug, this may not be very important but in the determination of the absolute toxicity, for example, the values obtained may not apply to the population in which we are interested, unless the number available is a random sample from the population concerned. For biological assays, however, since we are interested only in comparative experiments, the problem is mainly to allot the animals at random to the different groups, from the total number available.

Suppose we have to choose 4,6 and 5 animals respectively for 3 groups from a total of 25 animals. We may then mark numbers 1 to 25 on separate cards and shuffle them well, and note the numbers on the first 4 cards and allot them to group (a); allot the numbers on the next 6 cards to group (b) and so on. We may then arrange the numbers in serial order for convenience indicating the groups also, as for example

I_c *3a 4c 5b*

If the animals have already been numbered, the allotment can be immediately made. If numbering the animals is difficult, they can be taken out of the cage, one by one, counting their serial numbers and then allotted to the appropriate groups.

Instead of using cards, which may involve some personal or mechanical element, the ideal method for obtaining a random sequence would be to use random numbers which may be obtained from any of the published tables with instructions on their use.

When the number available in any batch is below 10 (or 20), a random permutation of the numbers 0,1,.....9 (or 0, 1,....., 19), will give a random sequence of numbers, if we omit the numbers which are not required.

When the number available is more than 20, the random sequence of numbers can be obtained from the general table of random numbers.

Ex.—To choose 3 groups (a,b,c) of 4,6 and 5 subjects respectively from a total number of 18 :

From the table of random permutations of 20 numbers (0-19), we get sequence :

2,16,6,18,7,10,15,3,8,14,5,9,13,1,11,4,12,17.

(Since we need only 18 numbers, 0 and 19 have been omitted).

Thus the numbers for the three groups will be

Group a: 2, 16, 6, 18.

Group b: 7, 10, 15, 3, 8, 14.

Group c: 5, 9, 13, 1, 11.

Arranging them in serial order we have

1c, 2a, 3b, 5c, 6a. . . . . ., 16a, 18a.

2.2 BIOLOGICAL ASSAY OF HUMAN ANTIHAEMOPHILIC FRACTION

The potency of human antihaemophilic fraction is determined by comparing the amount necessary to reduce the clotting time of a test mixture containing substances that cause clotting of blood with the amount of the Standard Preparation necessary to produce the same effect under the conditions of the following method of assay:

Standard Preparation and Unit

The Standard Preparation is a freeze-dried concentrate of human antihaemophilic factor or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

The Unit is the specific antihaemophilic factor contained in such an amount of the Standard Preparation as the Ministry of Health, Govt. of India may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Reagents

(1) Normal serum reagent : Collect normal human blood in a dry, sterile glass bottle, incubate at 37° for three hours, maintain at 4° overnight, remove the serum, dry from the frozen state, and keep in a vacuum desiccator over *phosphorous pentoxide*. Dissolve a quantity of the dried serum calculated to have been obtained from 1 ml of the serum in sufficient *imidazole buffer pH 7.4* to produce 10 ml and allow to stand at 4° for twenty-four to thirty-six hours.

(2) Phospholipid : Wash a quantity of normal human or bovine brain freed from meninges and blood vessels and macerate in a suitable blender. Weigh 1000 to 1300 g of the macerate and measure its volume (v ml). Extract with three quantities, each of 4v ml, of *acetone*, filter by suction, and dry the precipitate at 37° overnight. Extract the dried precipitate with two quantities, each of 2v ml, of a mixture of two volumes of *light petroleum (boiling range, 30° to 40°)* and three volumes of *light petroleum (boiling range, 40° to 60°)*, filtering each extract through a filter paper previously washed with the light petroleum mixture. Combine the extracts and evaporate to dryness at 45° at a pressure not exceeding 5 torr.

Dissolve the residue in 0.2v ml of *solvent ether* and allow to stand at 4° until a deposit forms. Centrifuge and evaporate the clear supernatant liquid under reduced pressure until the volume is about 100 ml per kg of the original macerate. Allow to stand at 4° until a precipitate forms (twelve to twenty-four hours), and centrifuge. To the clear supernatant liquid add five times its volume of *acetone*, centrifuge, discard the supernatant liquid, dry the precipitate and store protected from light in a vacuum desiccator.

(3) Phospholipid reagent : Suspend 0.125 g of *phospholipid* in 5 ml of *water*, shake, and stir until a uniform suspension is obtained. Prepare a dilution with *saline solution* that will give minimum clotting times consistent with the largest clotting time differences between consecutive dilutions of the Standard Preparation and the preparation being examined. The concentration usually lies between 50 and 250 µg per ml. The diluted suspension may be kept at −20° for six weeks.

(4) Clotting factor V solution : Prepare from fresh oxalated bovine plasma by fractionation at 4° with a saturated solution of *ammonium sulphate* prepared at 4°. Use the fraction precipitating between 38 per cent and 50 per cent saturation (which contains clotting factor V not significantly contaminated with clotting factor VIII), dialysed to remove ammonium sulphate and diluted with *saline solution* to give a solution containing between 10 per cent and 20 per cent of the amount of clotting factor V present in fresh normal human plasma.

Determine the clotting factor V content of the solution as follows: Prepare two dilutions in *imidazole buffer pH 7.4* to contain 1 volume of the solution being examined in 10 volumes and 20 volumes respectively. Test each dilution as follows : Mix 0.1 ml of each of *substrate plasma deficient in clotting factor V*, the dilution being tested, *thrombokinase extract*, and *0.025M calcium chloride*, and record as the clotting time the interval between the addition of the calcium chloride solution and the first indication of fibrin formation, which may be observed visually or by mechanical means.

Similarly determine the clotting times, in duplicate, for four dilutions of pooled normal human plasma in *imidazole buffer pH 7.4* containing one volume in 10 volumes (equivalent to 100 per cent of clotting factor V), 50 volumes (20 per cent), 100 volumes (10 per cent), and 1000 volumes (1 per cent), respectively.

To calculate the result, plot the mean of the clotting times for each dilution of human plasma on double cycle log/log paper against the equivalent percentage of clotting factor V and read the percentage of clotting factor V for the two dilutions of *clotting factor V solution* by interpolation from the curve. The mean of the two results is taken as the percentage of clotting factor V in the solution.

(5) Substrate plasma : Separate the plasma from 9 volumes of human or bovine blood collected in 1 volume of a 3.8 per cent w/v solution of *sodium citrate* or from 3.5 volumes of human or bovine blood collected in 1 volume of a solution containing 2 per cent w/v of *sodium acid citrate* and 2.5 per cent w/v of D-*glucose*. Substrate plasma may be prepared up to two days after collection of the blood.

(6) Substrate plasma deficient in clotting factor V : Preferably use congenitally deficient plasma or, alternatively, prepare as follows: Separate the plasma from human blood collected in one-tenth its volume of a 1.34 per cent w/v solution of *sodium oxalate* and incubate at 37° for twenty-four to thirty-six hours. This plasma should have a clotting time, when tested by the assay method given under *clotting factor V solution*, of seventy to one hundred seconds; if the clotting time is less than seventy seconds, incubate the plasma for a further twelve to twenty-four hours. Store, in small amounts, at −20° or below.

Method

Reconstitute the entire contents of one ampoule of the Standard Preparation with 1 ml of *water*; use immediately.

To the reconstituted Standard Preparation and the preparation being examined, add sufficient *imidazole buffer pH 7.4* to produce solutions containing between 0.5 and 2 Units per ml; these solutions are stable for fifteen minutes at 20°. Using a mixture of one volume of a 3.8 per cent w/v solution of *sodium citrate* and five volumes of a 0.85 per cent w/v solution of *sodium chloride* as the diluent, make from the solutions three successive two-fold dilutions in the range 1 in 16 to 1 in 256 so that all the clotting times are between seventeen and thirty-five seconds;

the dilutions must be accurately made and used immediately.

Introduce into each of six glass incubation tubes, 75 × 10 mm, 0.1 ml of each of *clotting factor V solution*, *phospholipid reagent*, and *normal serum reagent*. To the first tube add 0.1 ml of the highest dilution of the Standard Preparation, place the tube in a water-bath at 37° , add 0.1 ml of *0.05M calcium chloride* and start a stop-watch. During the next minute add 0.1 ml of the second highest dilution of the standard to a second tube, place it in the water-bath, and add 0.1 ml of *0.05M calcium chloride* at one minute by the stop-watch. Repeat the procedure with the lowest dilution of the standard and the highest to lowest dilutions of the preparation being examined so that the calcium chloride solution is added at two, three, four and five minutes by the stop-watch, respectively.

Place in a water-bath at 37° twelve glass tubes each containing 0.2 ml of *0.025M calcium chloride* and a further tube containing about 3 ml of *substrate plasma*. At fourteen minutes, forty seconds by the stop-watch, transfer 0.1 ml of the mixture from the first incubation tube to one of the tubes containing 0.2 ml of the *0.025M calcium chloride solution* and mix. At fifteen minutes add 0.2 ml of the warmed substrate plasma and, using a second stop-watch record as the clotting time the interval between the addition of the substrate plasma and the first indication of fibrin formation, which may be observed visually or by mechanical means. Repeat the procedure with the other incubation tubes at one minute intervals and carry out a second series of determinations at twenty-one to twenty-six minutes. The period of incubation should, if necessary, be adjusted so that the clotting times recorded in the corresponding tests in the two series of determinations do not differ by more than 5 per cent, showing that a stable plateau of prothrombin activator formation has been reached.

Carry out a blank determination using in place of the preparation being examined, an equal quantity of a mixture of one volume of a 3.8 per cent w/v solution of *sodium citrate* and five volumes of a 0.85 per cent w/v solution of *sodium chloride*. The result of the assay is invalid if the clotting time in the blank determination is less than forty seconds.

Calculate the result of the assay by standard statistical methods.

2.3 BIOLOGICAL ASSAY OF HEPARIN SODIUM

The potency of heparin sodium is determined by comparing the concentration necessary to prevent the clotting of sheep plasma with the concentration of the Standard Preparation of heparin sodium necessary to give the same effect under the conditions of the following method of assay.

Standard Preparation and Unit

The Standard Preparation is the freeze-dried sodium salt of the purified active principle from bovine intestinal mucous membrane or any other suitable preparation, the potency of which has been determined in relation to the International Standard

The Unit is the specific activity contained in 7.7µg of the Standard Preparation and is the same as the International Unit. 1 mg contains 130 Units

Reagents

(1) Prepared plasma : Collect blood from sheep directly into a vessel containing 8 per cent w/v solution of *sodium citrate* in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Immediately centrifuge and pool the separated plasma. To a 1-ml quantity of the pooled plasma in a clean test-tube add 0.2 ml of a 1 per cent w/v solution of *calcium chloride* and mix. The plasma is suitable if a solid clot forms within five minutes.

(2) Solution of standard preparation : Determine by preliminary trial, if necessary, approximately the minimum quantity of the Standard Preparation of heparin sodium which, when added in a 0.8 ml of *saline solution*, maintains fluidity in 1 ml of *prepared plasma* for one hour after the addition of 0.2 ml of a 1 per cent w/v solution of *calcium chloride*. On the day of the assay prepare a solution of the Standard Preparation such that it contains, in each 0.8 ml of *saline solution*, the above-determined quantity of the Standard Preparation.

(3) Test solution : Weigh accurately about 25 mg of the preparation being examined and dissolve in sufficient *saline solution* to give a concentration of 1 mg per ml and dilute to a concentration estimated to correspond to that of the solution of Standard Preparation.

Method

To very clean test-tubes (of length 150 mm and diameter 16 mm) add graded amounts of the *solution of standard preparation*, selecting the amounts so that the largest dose does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube add sufficient *saline solution* to make the total volume 0.8 ml. Add 1.0 ml of *prepared plasma* to each tube. Then add 0.2 ml of a 1 per cent w/v solution of *calcium chloride*, note the time, immediately stopper each tube with a suitable stopper, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the *test solution*, completing the entire process of preparing and mixing the tubes of both the *solution of standard preparation* and the *test solution* within twenty minutes after the addition of the *prepared plasma*. Exactly one hour after the addition of the calcium chloride, determine the extent of clotting in each tube, recognising three grades between zero and full clotting.

The dilution of the *test solution* which contains heparin sodium in the same concentration as the dilution of the *solution of standard preparation* is that contained in the series of dilutions which show the same degree of clotting as the series of dilutions of the *solution of standard preparation*. If the degree of clotting observed in the series of dilutions of the *solution of standard preparation* lies between that observed in two of the series of the dilutions of the sample being tested, the potency of the latter is estimated. If there is no such correspondence between the degrees of clotting produced by the *solution of standard preparation* and any of the dilutions of the sample being tested, new dilutions of the latter are prepared and the assay is repeated.

Limits of error – The limits of error (P = 0.99) attainable with this test are:

80 and 120 per cent, with one determination;

86 and 114 per cent, with two determinations;

90 and 110 per cent, with three determinations;

92 and 108 per cent, with four determinations;

2.4 DETERMINATION OF AMYLASE ACTIVITY

Weigh accurately a quantity equivalent to 100 Units of amylase activity and triturate with 200 ml of *buffer solution pH 6.0* (for bacterial amylase) or of *acetate buffer pH 5.0* (for fungal amylase) and add sufficient *buffer solution pH 6.0* or *acetate buffer pH 5.0* to produce 1000.0 ml. Dilute 10.0 ml to 100.0 ml with *buffer solution pH 6.0* or *acetate buffer pH 5.0* to give the test solution; filter if necessary (1 ml of the *test solution* should be capable of digesting about 10 mg of dry soluble starch). Into each of six stoppered test-tubes add 5.0 ml of *starch substrate* (without touching the sides of the test-tube). Place these test-tubes in a water-bath maintained at 40° ± 0.1° When the temperature of the solution in the tubes has reached 40°, add 0.35 ml, 0.4 ml, 0.45 ml, 0.5 ml, 0.55 ml and 0.6 ml of the *test solution* to each of the test-tubes marked 1 to 6 respectively and record the time of addition. Mix thoroughly and replace the tubes in the water-bath. After exactly sixty minutes remove the tubes and cool rapidly in cold water. Add to each tube 0.05 ml of *0.02 N iodine* and mix well. Note the tube containing the lowest volume of *test solution* which does not show a bluish or violet tinge (if there is doubt, warm the solution slightly, when the colour distinction is prominent). From this volume calculate the number of grams of dry soluble starch digested by 1.0 g of the sample. This represents the number of Units of amylase activity per g.

2.5 DETERMINATION OF PROTEOLYTIC ACTIVITY

Weigh accurately 4.0 g of *purified casein* and dissolve in about 90 ml of *water* containing 3 ml of *N sodium hydroxide*, adjust the pH of the solution to 8.7 and add sufficient *water* to produce 100.0 ml. Triturate 0.5 g of the substance being examined with *water* and add sufficient *water* to produce 300.0 ml to give the test solution. Dilute 15.0 ml of the casein solution with 30 ml of *water*, warm to 55° and add 10.0 ml of the unfiltered *test solution*. Heat rapidly to 55° and keep at this temperature for twenty minutes. Cool rapidly to room temperature. Dilute a further portion of 15.0 ml of the casein solution with 30 ml of *water*, add 10.0 ml of the unfiltered *test solution*, previously boiled and cooled, heat rapidly to 55° and keep at this temperature for twenty minutes. Cool to room temperature. To each solution add 0.75 ml of a 0.1 per cent w/v solution of *phenolphthalein* and 10 ml of *formaldehyde solution*. Titrate each solution with *0.1 N sodium hydroxide* until the colour of the solution matches that produced by mixing 10 ml of *buffer solution pH 8.7* and 0.15 ml of a 0.1 per cent w/v solution of *phenolphthalein*. Note the difference between the two titrations.

2.6 ASSAY OF HYALURONIDASE

Reagents

(1) Acetate buffer solution : Dissolve 14 g of *potassium acetate* and 20.5 ml of *glacial acetic acid* in sufficient *water* to produce 1000 ml.

(2) Diluent for hyaluronidase solutions : Mix 250 ml of *phosphate buffer solution* with 250 ml of *water* and, within two hours before use, dissolve 330 mg of *hydrolysed gelatin* in the mixture.

(3) Hyaluronate solution : On the day of the assay, dilute 1 volume of *sodium hyaluronate stock solution* with 1 volume of *phosphate buffer solution*.

(4) Hydrolysed gelatin : Dissolve 50 g of *gelatin* in 1000 ml of *water*, heat in saturated steam at 121° for ninety minutes and dry from the frozen state.

(5) Phosphate buffer solution : Dissolve 2.5 g of *sodium dihydrogen phosphate*, 2.523 g of *disodium hydrogen phosphate* and 8.2 g of *sodium chloride* in sufficient *water* to produce 1000 ml.

(6) Serum solution : On the day of the Assay, dilute 1 volume of *serum stock solution* with 3 volumes of *acetate buffer solution* and adjust to room temperature.

(7) Serum stock solution : Dilute 1 volume of cattle or horse serum (native or reconstituted with *water* to its original volume) with 9 volumes of *acetate buffer solution*. Adjust the pH to 3.1 with *4 N hydrochloric acid* and allow to stand for eighteen to twenty-four hours. Store at 0° to 4° and use within thirty days.

(8) Sodium hyaluronate stock solution : Prepare a stock solution to contain in each ml 500 µg of *sodium hyaluronate*, previously dried over *phosphorous pentoxide* under reduced pressure for forty-eight hours. Store at a temperature below 0° and use within thirty days. Do not

keep the sodium hyaluronate over *phosphorous pentoxide* indefinitely.

(9) Sodium hyaluronate : Cut human umbilical cords, freed from blood and stored under *acetone*, into segments 2 cm long and wash with 10 volumes of *acetone*. Wash by soaking in three quantities, each of 10 volumes, of *water* for two, two, and twenty-four hours. Mince the cords, add an equal volume of *water*, adjust the pH to 2.0 with *2 N hydrochloric acid*, add 3 g of *pepsin* for each kg of cords, cover with a layer of *toluene*, and incubate at 37° for twenty-four hours, maintaining the pH at 2.0. Adjust the pH to 7.4 by the cautious addition of a 40 per cent w/v solution of *sodium hydroxide*, add 5 g of *trypsin* for each kg of cords, and incubate at 37° for twenty-four hours. Centrifuge the aqueous layer, decant the clear liquid, cool to 5°, adjust the pH to 2.0 with a mixture of equal volumes of *hydrochloric acid* and *water*, and add, with stirring, two volumes of *alcohol*. Separate the precipitate by centrifuging, suspend it in 300 ml of *water* for each kg of cords, and dialyse against running tap-water for twenty-four hours. To each litre of suspension add 660 ml of *chloroform*, 340 ml of *amyl alcohol*, and 200 ml of a solution containing 30 per cent w/v of *sodium acetate* and 16 per cent w/v of *glacial acetic acid* in *water*. Shake vigorously for ten minutes, centrifuge, and treat the separated aqueous phase repeatedly with further portions of the chloroform-amyl alcohol mixture until no precipitate is formed at the interface. To the aqueous phase add two volumes of *alcohol*, centrifuge, dissolve the residue in *water*, dialyse against running tap-water for twenty-four hours and then against several changes of *water* for twenty-four hours; dry the product from the frozen state.

Standard Preparation and Unit

The Standard Preparation is a quantity of the dried active principle from ox testes, diluted with *lactose* (supplied in ampoules containing 20 mg tablets).

The Unit is the specific activity contained in such an amount of the Standard Preparation as the Ministry of Health, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use. The Unit is contained in 0.10 mg of the Standard Preparation at present in use.

Preparation of solution of the standard preparation : Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold *diluent for hyaluronidase solutions* to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

Method

Dissolve the contents of a single container of the sample being tested by adding cold *diluent for hyaluronidase solutions* directly to the container. Dilute the solution with cold *diluent for hyaluronidase solutions* so that the *extinctions* of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows:

To each of twelve test-tubes, 16 mm in diameter and 100 mm in length, add 0.50 ml of *hyaluronate solution* and, respectively and in duplicate, 0.50, 0.40, 0.30, 0.20, 0.10 and 0.00 ml of *diluent for hyaluronidase solutions*. If quantities of the solution of the Standard Preparation other than those indicated below are used, change the quantities of *diluent for hyaluronidase solutions* accordingly. Add to the tubes at intervals of thirty seconds, 0.00, 0.10, 0.20, 0.30, 0,40, and 0.50 ml of solution of the Standard Preparation, respectively and in duplicate, making the final volume in each tube 1.00 ml, mixing the contents by gentle shaking, and placing each tube in a water-bath maintained at 37° ± 0.20°. After exactly thirty minutes, remove each tube in order from the water-bath at intervals of thirty seconds and immediately add 4.0 ml of *serum solution*; shake and allow to stand at room temperature for thirty minutes; shake again and determine the *extinction* at 640 nm, Appendix 5.15 A. Repeat the operation using 0.50 ml of *phosphate buffer solution* in place of the *hyaluronate solution* and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected *extinctions* for each level against the potency.

To each of six test-tubes, 16 mm in diameter and 100 mm in length, add 0.50 ml of *hyaluronate solution* and sufficient *diluent for hyaluronidase solutions* so that the final volume in each tube after the addition of the solution of the sample being tested is 1.00 ml. Add to each tube at intervals of thirty seconds, sufficient of the solution of the sample being tested so that the tubes contain about 0.30, 0.50, and 0.70 Units, respectively and in duplicate, shaking each tube gently and continuing as described in the preceding paragraph commencing at the words 'placing each tube in a water-bath'.

The potency of the sample being tested is given by the mean of the six values read from the reference curve.

2.7 BIOLOGICAL ASSAY OF CHORIONIC GONADOTROPHIN

The potency of chorionic gonadotrophin is determined by comparing its effect in enlarging the prostate glands of immature rats with that of the Standard Preparation of chorionic gonadotrophin under the conditions of the following method of assay.

Standard Preparation and Unit

The Standard Preparation is a dried extract of the active principle from human urine of pregnancy or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

The Unit is the specific gonadotrophic activity contained in 0.0001 g of the Standard Preparation.

Method

Use male rats approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four litter mates are available, allot one litter mate from each set at random to each group and make according to litter.

Choose two doses of the Standard Preparation and two of the preparation being tested such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a positive response in all the rats. As an initial approximation, doses of 0.75 and 1.5 Units may be tried although the dose will depend on the sensitivity of the animals used. Dissolve each dose in 0.2 ml of *saline solution* and administer by subcutaneous injection on three consecutive days at the same time each day. On the fourth day, about twenty-four hours after the last injection, kill the rats and remove the prostate glands from each animal and weigh immediately. Calculate the result of the assay by standard statistical methods.

2.8 BIOLOGICAL ASSAY OF CORTICOTROPHIN

The potency of corticotrophin is determined by comparing its adrenal ascorbic acid depletion property with that of the Standard Preparation of corticotrophin under the conditions of a suitable method of assay. When the material being tested is intended for administration by subcutaneous or intramuscular injection, Method I is used and when it is intended for administration by intravenous injection, Method II is used.

Standard Preparation and Unit

The Standard Preparation is a quantity of dry corticotrophin. The Unit is the specific activity contained in such an amount of the Standard Preparation as the Ministry of Health, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use. The Unit is contained in 0.88 mg of the Standard Preparation at present in use.

Methods

Method I : Use rats of either sex weighing between 100 and 200 g; the range of weights in any one test should be kept as small as possible and should, in any case, not exceed 15 g. Keep the rats under uniform conditions for at least a week before the test. On the day before the test, weigh and hypophysectomise the rats. After the operation allow access to a 5 per cent solution of *dextrose (anhydrous)* in *saline solution* in addition to rat diet and water and keep the animals in a quiet room at a constant temperature between 24° and 27°. Carry out the rest between eighteen and thirty-six hours after hypophysectomy.

On the day of the test reweigh the animals and assign them at random to six groups of eight to ten animals. Choose three doses of the Standard Preparation and three of the preparation being tested such that the smallest dose produces some depletion, and the largest dose does not produce maximum depletion of the adrenal ascorbic acid content. Administer, in random order by subcutaneous injection, doses adjusted to the body weight of the animals. Doses of the order of 1.0, 0.5, and 0.25 Unit per 100 g of body weight are usually suitable. Unless otherwise stated in the monograph, dissolve the preparations in *0.01N hydrochloric acid* and make subsequent dilutions with *water* containing 15 per cent w/v of *gelatin*; the solutions should be injected within four hours of preparation.

Three hours after injection, remove both adrenal glands from the anaesthetised rat, free them from extraneous tissue, and weigh, the operation being carried out as quickly as possible to avoid weight losses. Kill the rat and examine for completeness of hypophysectomy.

Homogenise the pair of adrenal glands in a freshly prepared 2.5 per cent w/v solution of *metaphosphoric acid*, and adjust the volume to 10 ml with the same solution. Allow the homogenate to stand for thirty minutes and centrifuge. Add 7 ml of the clear supernatant liquid to a freshly prepared mixture of 7 ml of a 4.53 per cent w/v solution of *sodium acetate* adjusted to pH 7 by the addition of *5N acetic acid*, 3 ml of *water*, and 2 ml of *standard 2,6-dichlorophenolindophenol solution*. Thirty seconds after mixing, measure the *extinction* of the resulting solution at the maximum at about 520 nm, Appendix 5.15 A. Calculate the weight of ascorbic acid from a standard curve prepared by treating suitable aliquots of a solution of L-*ascorbic acid* in a 2.5 per cent w/v solution of *metaphosphoric acid* by the same process, and express in mg per 100 g of adrenal gland. Calculate the result of the assay by standard statistical methods.

Method II : Follow Method I with the following modifications:

(i) Administer the Standard Preparation and the preparation being tested by intravenous injection in *saline solution* without the addition of gelatin and acidify by the addition of 0.25 per cent v/v of *5N acetic acid*.

(ii) Doses of the order of 1.0, 0.5, and 0.25 milliunit per 100 g of body weight are usually suitable.

(iii) Remove the adrenal glands between fifty-five and sixty-five minutes after injection.

Limits of error – The fiducial limits of error of the estimated potency (P = 0.95) are not less than 64 per cent and not more than 156 per cent of the stated potency.

2.9 BIOLOGICAL ASSAY OF INSULIN

The potency of a sample of insulin is determined by comparison of the hypoglycaemic effect it produces with that

produced by the Standard Preparation of insulin under the conditions of the assay.

Standard Preparation

The Standard Preparation is pure, dry, crystalline insulin containing 24.0 Units per mg or any other suitable preparation the potency of which has been determined in relation to the International Standard.

Preparation of Solution of the Standard Preparation

Prepare a solution of the Standard Preparation containing 40 Units in 1 ml by dissolving a suitable quantity in *saline solution* acidified with *hydrochloric acid* to pH 2.5 and containing sufficient of a substance to prevent the growth of micro-organisms. Store the solution at a temperature between 2° and 8°, protected from freezing. The solution may be used over a period not exceeding six months from the date of preparation.

Methods

Any of the following recommended methods may be followed:

Method I : Use not less than 96 mice, keeping the range of weight in any test as small as possible and not exceeding 5g, which have been fed on an adequate diet. Prepare the mice for the assay by deprivation of food not less than two and not more than twenty-hours preceding the test. Distribute the mice at random into four equal groups. Immediately before use make two dilutions of the solution of the Standard Preparation and two dilutions of the preparation being tested as follows: Make two dilutions of the solution of the Standard Preparation with *saline solution* acidified with *hydrochloric acid* to pH 2.5, to obtain solutions containing 60 milliunits and 30 milliunits of insulin per ml. Make the dilutions of the preparation being examined so that, if the assumption of potency is correct, solutions containing amounts of insulin equivalent to the dilutions of the Standard Preparation are obtained.

Inject each of the prepared solutions subcutaneously into one group of mice, using the same volume, which should not exceed 0.5 ml, for each mouse. Keep the mice at a uniform temperature, between 29° and 35°, either in an air incubator with a transparent front with the mice in transparent containers within, or in a series of small boxes two-thirds immersed in a water-bath at a suitable temperature, allowing for adequate ventilation of all the containers. Arrange the test so that the containers for all four groups are distributed evenly over the whole incubator or water-bath. Observe the mice for one and a half hours after injection and record the number of mice which are dead or convulsed, or which lie still for more than two or three seconds when placed on their backs.

Calculate the result of the assay by standard statistical methods.

The surviving animals may be used again upto three times and if necessary supplemented with fresh mice, care being taken to distribute them equally over the four groups of animals.

Method II : Use not less than twenty-four healthy rabbits each weighing not less than 1.8 kg. Keep the rabbits in the laboratory for not less than one week before use in the assay, maintained on a uniform diet. Distribute the rabbits at random into four equal groups and mark for identification within each group. Approximately twenty hours before the test provide each rabbit with an amount of food that will be consumed within six hours. Follow the same feeding schedule before each test day. During the test withhold all food and water until after the final blood sample is taken. Handle the rabbits with care to avoid undue excitement.

Immediately before use make two dilutions of the solution of the Standard Preparation, containing 1.0 Unit and 2.0 Units of insulin per ml, respectively, and two dilutions of the preparation being examined which, if the assumption of potency is correct, contain amounts of insulin equivalent to the dilutions of the Standard Preparation. Use as diluent a solution containing 0.1 to 0.25 per cent w/v of either *m-cresol* or *phenol* and 1.4 to 1.8 per cent w/v of *glycerin* and acidify with *hydrochloric acid* to a pH between 2.5 and 3.5.

Inject each of the prepared solutions subcutaneously into one group of rabbits, using the same volume, which should usually be between 0.30 and 0.50 ml. for each rabbit, the injections being carried out according to a randomised block design. Preferably on the following day, but in any case not more than one week later, administer each solution to a second group of rabbits following a twin cross-over design. One hour, and two and half hours, after each injection take a suitable blood sample from the marginal ear vein of each rabbit. Determine the blood glucose concentration of each sample by either of the following two methods:

(A) Pipette into separate, suitable vessels 0.8 ml of each blood sample and 0.8 ml each of standard solution of *dextrose (anhydrous)* containing, respectively, the following concentrations of anhydrous dextrose: 0.25, 0.50, 0.75, 1.0 and 1.25 mg per ml. Into each vessel pipette 2.4 ml of *saline solution* and mix. Place each vessel in a water-bath maintained at 38° and subject the diluted blood to dialysis across a semipermeable membrane for a sufficient time for a definite proportion of the dextrose to pass through the membrane into a *saline solution* containing 0.06 per cent w/v of *potassium ferricyanide* and 2.0 per cent w/v of *sodium carbonate*. Pipette a known volume of a 0.5 per cent w/v solution of *potassium cyanide* and heat at a temperature of 95° for five minutes. Cool to 40° and determine the *extinction* of the resulting solution at 420 nm, Appendix 5.15 A, using as blank *water* treated in the same manner. Calculate the content of dextrose from the *extinction* obtained by carrying out the operation using the standard

solutions of dextrose instead of the diluted blood being examined.

Calculate the result of the assay by Standard Statistical Methods.

(B) Pipette 0.1 ml of the blood sample into a centrifuge tube, add 3.5 ml of *water*, 0.2 ml of *0.3 N barium hydroxide* and 0.2 ml of a 5 per cent w/v solution of *zinc sulphate*. Shake well and centrifuge. Pipette 1.0 ml each, of the supernatant liquid, 1.0 ml of *water* and 1.0 ml of *standard glucose solution* into three test-tubes, add to each 1.0 ml of *water* and 2.0 ml of *alkaline copper solution* and heat in a boiling water-bath for ten minutes. Cool quickly for one minute, add 1.0 ml of *arsenomolybdate solution* and make upto 10.0 ml with *water*. Measure the *extinction* of a 1-cm layer of each of the solutions at the maximum at about 680 nm, Appendix 5.15 A, and calculate the content of glucose in the samples of blood.

Calculate the result of the assay by Standard Statistical Methods.

2.10 TEST FOR PROLONGATION OF INSULIN EFFECT

The prolongation of the hypoglycaemic effect produced by modified preparations of insulin is compared with the hypoglycaemia produced by the Standard Preparation of insulin in guinea-pigs or rabbits.

Distribute at random into two equal groups not fewer than ten animals from a healthy colony. House the animals singly and deprive them of food (but not water) from eighteen hours before the completion of the test. At the beginning of the test determine the mean blood-glucose level of each group. Inject subcutaneously the animals in one group with the preparation being tested and the animals in the other group with a *solution of the Standard Preparation* in *saline solution* acidified with *hydrochloric acid* to pH 2.5, prepared to have the nominal potency of the preparation being tested; inject both preparations without further dilution in equivalent amounts corresponding to the body weights of the animals.

At one, two, four, and six hours after injection, determine the mean blood-glucose level of each group of animals by a suitable method such as that described under Method II of the *biological assay of insulin*, Appendix 2.9.

2.11 TEST FOR INSULIN IN SOLUTION

Carry out the *biological assay of insulin*, Appendix 2.9, with the following modifications:

(1) Use two groups of randomly selected animals,each group consisting of either twenty-four mice (Method I) or four rabbits (Method II).

(2) Inject one group of animals with an appropriate dilution of the *solution of the Standard Preparation* (when Method I is used, an appropriate dilution is one which will produce responses in about half of the mice). Suitable concentrations are 60 milliunits of insulin per ml for Method I and 1.0 to 2.0 Units of insulin per ml for Method II.

(3) Inject the other group of animals with the clear liquid obtained by centrifuging or filtering the preparation being examined (Liquid A), or a dilution thereof, making the unitage and volumes of the standard and test solutions injected equal on the assumption that Liquid A contains 2.5 per cent of the stated activity.

If Method I is used, the number of mice which respond in the group receiving Liquid A is less than the number which respond in the group receiving the Standard Preparation.

If Method II is used, the average reduction in blood glucose in the rabbits receiving Liquid A is not greater than that in the rabbits receiving the Standard Preparation.

2.12 BIOLOGICAL ASSAY OF OXYTOCIN INJECTION

The potency of oxytocin injection is determined by comparing its activity with that of the Standard Preparation of oxytocin under the conditions of the following method of assay.

Standard Preparation and Unit

The Standard Preparation is a freeze-dried preparation of oxytocin with albumin and citric acid or any other suitable preparation, the potency of which had been determined in relation to the International Standard.

The Unit is the specific oxytocin activity corresponding to that yielded by 0.0005 g of the Standard Preparation.

Extract of standard preparation : Proceed as described under *biological assay of vasopressin injection*, Appendix 2.13.

Method

(1) Test animals : Use female rats in dioestrus, weighing between 120 and 200 g or nulliparous, anoestrus female guinea-pigs weighing between 175 g and 350 g.

(2) Method of comparison : Sacrifice a rat or guinea-pig by a blow on the head or by decapitation and immediately remove the entire uterus from the body. Suspend one horn of the uterus in a chamber, containing freshly prepared *Locke-Ringer's solution*, of an apparatus used for recording the activity of isolated smooth muscle. Maintain the solution surrounding the uterus at a temperature between 37° and 38° with a variation of not more than 0.1° during the course of the test or alternatively at any suitable temperature at which spontaneous contractions of the uterus are abolished and the preparation maintains its sensitivity. Oxygenate the solution with a mixture of 95 per cent of *oxygen* and 5 per cent of *carbon dioxide*. and

record the contractions of the muscle on a suitable recorder using an isotonic and linear lever or transducer. Record the contractions produced by the addition to the bath of two doses of the Standard Preparation suitably diluted with the above solution. The doses should be such as to produce clearly discriminated submaximal contractions. When the contractions are complete, replace the solution in the bath by fresh solution to relax the muscle. Dilute the preparation being tested so as to obtain responses on the addition of two doses similar to those obtained with the Standard Preparation. The ratio between the two doses of the preparation being tested should be the same as that between the two doses of the Standard Preparation, and this ratio should be kept constant throughout the assay

The two doses of Standard Preparation and the two doses of the preparation under test should be given in a random order, and at least four responses to each should be recorded. Instead of completely randomising the order of administration some form of restricted design may be used, provided that this permits the elimination of the effect of changes in the sensitivity of the preparation with time from the estimate of potency obtained from the assay. The doses should be added at regular intervals of three to five minutes depending upon the rate of recovery of the muscle.

Measure all the responses and calculate the result of the assay by Standard Statistical Methods.

2.13 BIOLOGICAL ASSAY OF VASOPRESSIN INJECTION

The potency of vasopressin injection is determined by comparing its activity with that of the Standard Preparation of vasopressin under the conditions of the following method of assay:

Standard Preparation and Unit

The Standard Preparation is a dried acetone extract of the posterior lobes of fresh pituitary bodies of oxen or any other suitable preparation, the potency of which has been determined in relation to that of the International Standard. It may be stored in sealed ampoules or in a desiccator "in vacuo" over *phosphorus pentoxide*.

The unit is the specific pressor activity corresponding to that yielded by 0.0005 g of the Standard Preparation.

Extract of the standard preparation : A portion of the dry powder, corresponding to about 20 Units, is transferred rapidly from the sealed ampoule or the desiccator to a weighing bottle, and the bottle is at once closed. The powder is weighed. It is washed into a dry, hard-glass boiling-tube with one-half as many ml of a mixture of 0.25 ml of *glacial acetic acid* and sufficient *water* to produce 100 ml as there are Units present in the quantity of powder taken. The top of the boiling-tube is plugged with cotton wool and the tube is placed for five minutes in briskly boiling water. The tube is quickly cooled and the liquid is filtered through a dry filter paper into another hard-glass tube. The filtrate is an extract of the Standard Preparation, or of the equivalent laboratory standard preparation, and contains 2 Units per ml. The filtrate is distributed into a series of sealed ampoules and sterilised by being placed in boiling water for three minutes. It is stored at 0° and remains unchanged in activity for six months. It must not be used as a standard later than six months after preparation. It is diluted immediately before use.

Methods

Use either of the following suggested methods:

Method I : Anaesthetise a male albino rat weighing about 300 g by the subcutaneous injection of a solution of *ethyl carbamate* or other anaesthetic which allows maintenance of a uniform blood pressure. Forty-five to sixty minutes later, tie the rat to the operating table by its hind legs, the front legs not being secured. Cannulate the trachea with a short glass or polyethylene tube of about 2.5 mm external diameter and dissect one carotid artery ready for cannulation. Then cannulate the femoral vein close to the inguinal ligament. Retract the abdominal muscles to expose the inguinal ligament. Retract the superficial pudendal vein to one side and dissect the femoral vein towards the inguinal ligament from the corresponding artery (when dissecting, a deep branch reaching the femoral vein must be found and tied off, otherwise bleeding may occur during cannulation). Tie a short polyethylene cannula of about 1 mm external diameter into the femoral vein by two ligatures and join by a short piece of rubber tubing to a 1-ml burette with an attached thistle funnel containing warm *saline solution*. Firmly fix a wet cotton swab to this thigh so as to cover the incision and cannula. At this stage inject, through the venous cannula, 200 Units of *heparin*, dissolved in *saline solution*, per 100 g of body weight. Then tie in the carotid cannula of about 1 mm external diameter and connect by a column of *saline solution* with a suitable pressure measuring device such as a mercury manometer of about 2 to 3 mm internal diameter.

The central and peripheral nervous system including both vagi and associated sympathetics is left intact. No artificial respiration is necessary. Taking care that no air is injected, inject all solutions through the venous cannula by means of a 1-ml syringe graduated in 0.01 ml and wash in with 0.2 ml of *saline solution* from the burette.

To produce a constant basal pressure of about 50 torr and to reduce or abolish the volume effect due to the injection of the solution, a suitable hypotensive agent such as phenoxybenzamine hydrochloride may be administered into the tail vein of the rat eighteen hours before anaesthetisation. Dilute the extract of the Standard Preparation and the preparation being tested with *saline solution* so that the volume to be injected is not less than 0.1 ml and not more than 0.5 ml.

Choose two doses of the Standard Preparation such that the elevation of the blood pressure is about 30 torr for the

lower dose and about 50 torr for the higher dose, the ratio of low to high dose being determined by the response and usually being 3 to 5. As an initial approximation doses of 6 and 10 milliunits may be tried. Choose two doses of the preparation being tested with the same inter-dose ratio, matching the effects of the dose of the Standard Preparation as closely as possible. Inject doses at intervals of about ten minutes. The two doses of Standard Preparation and the two doses of the preparation under test should be given in a random order, and at least four responses to each should be recorded. Instead of completely randomising the order of administration, some form of restricted design may be used, provided that this permits the elimination of the effect of changes in the sensitivity of the preparation with time from the estimate of potency obtained from the assay. The doses should be added at regular intervals of three to five minutes depending upon the rate of recovery of the muscle. Record the maximum rise in blood pressure in response to each dose.

Calculate the result of the assay by Standard Statistical Methods.

Method II : Anaesthetise a full-grown, healthy cat with a volatile anaesthetic and insert a tracheal tube. Expose the spinal cord from behind by removing a part of the second cervical vertebra and divide it. Destroy the brain by passing a suitable instrument through the foramen magnum and then plug with a cork. Immediately start artificial respiration through the tracheal tube and leave the animal aside for an hour to remove the anaesthetic effect. Arrange to obtain a continuous record of the blood pressure from the carotid artery. Arrange for intravenous injections through a suitable cannula approximately 1 mm in external diameter inserted in a femoral or jugular vein.

Keep the animal warm during its preparation and during the assay.

Keep the initial pressure approximately constant and equivalent to 50 to 100 torr. Taking care that no air is injected, inject all solutions through the venous cannula by means of a syringe and wash in with *saline solution* contained in a burette attached to the cannula by a short piece of rubber tubing.

Choose doses of the Standard Preparation and of the preparation being tested which give matching effects and make repeated injections at regular intervals of time. A dose of 0.05 to 0.1 Unit at intervals of thirty minutes will give constant response of suitable size. The doses of the Standard Preparation and of the preparation being tested should be given in a random order. Record the maximum rise in blood pressure in response to each dose.

Calculate the result of the assay by Standard Statistical Methods.

2.14 BIOLOGICAL ASSAY OF DIPHTHERIA ANTITOXIN

The potency of diphtheria antitoxin is determined by comparing the dose necessary to protect guinea-pigs or other suitable animals against the effect of a fixed dose of diphtheria toxin with the dose of the Standard Preparation of diphtheria antitoxin necessary to give the same protection. For this purpose a suitable preparation of diphtheria toxin is required to be used as a test toxin. The test dose of the toxin is determined in relation to the Standard Preparation. The potency of the preparation being examined is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation and Unit

The Standard Preparation is a quantity of dried diphtheria antitoxin. The Unit is the specific neutralising activity for diphtheria toxin contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

(1) Test toxin : Prepare diphtheria toxin by filtering through a bacteria-proof filter the fluid medium in which a toxigenic strain of *Corynebacterium diphtheriae* has grown.

(2) Selection of test toxin : In selecting a toxin for use as the test toxin determine the following:

Lr/100 dose – This is the smallest quantity of the toxin which, when mixed with 0.01 Unit of antitoxin and injected intracutaneously into guinea-pigs or rabbits causes a small, characteristic reaction at the site of the injection.

Minimal reacting dose – This is the smallest quantity of toxin which, when injected intracutaneously into guinea-pigs or rabbits, causes a small, characteristic reaction at the site of injection.

A suitable toxin is one which has at least 200 minimal reacting doses in the Lr/100 dose. The test toxin is allowed to stand for some months before being used for the assay of samples of antitoxin. During this time its toxicity declines and the Lr/100 dose may be slightly increased. When experiment shows that the Lr/100 dose is constant, the test toxin is ready for use, and may be used for a long period. Determine the minimal reacting dose and the Lr/100 dose at frequent intervals. Store the test toxin in the dark between 0° and 5°. Maintain its sterility by the addition of *toluene* or other bactericide which does not cause a rapid decline in specific toxicity.

(3) Determination of test dose of toxin (Lr/100 dose) : Prepare a solution of the Standard Preparation with a *saline solution* such that 1 ml contains 0.1 Unit. Prepare mixtures such that 2 ml of each mixture contains 1 ml of the dilution of the Standard Preparation (0.1 Unit) and one of a series of graded volumes of the test toxin. Dilute each mixture with *saline solution* to the same final volume (2 ml). Allow the mixtures to stand for not less

than fifteen minutes and not more than sixty minutes and inject them intracutaneously into the shaven or depilated flanks of the animals. Inject a dose of 0.2 ml of each mixture at suitably spaced sites into the skin of the animal. Observe the animals thereafter for two days.

The test dose (Lr/100) of the toxin is the amount present in 0.2 ml of that mixture which causes at the site of injection a small, characteristic reaction in the skin of the guinea-pig or rabbit. Mixtures containing larger amounts of toxin cause a larger reaction and necrosis and mixtures containing smaller amounts of toxin cause no reaction.

(4) Determination of the potency of the antitoxin : Dilute the test toxin with *saline solution* so that 1 ml contains ten times the test dose. Prepare mixtures such that 2 ml of each mixture contains 1 ml of the solution of test toxin and one of a series of graded volumes of the preparation being examined. Prepare a further mixture such that 2 ml contains 1 ml of the solution of test toxin and 0.1 Unit of antitoxin. Dilute each mixture with *saline solution* to the same final volume (2 ml). Allow the mixtures to stand for not less than fifteen minutes and not more than sixty minutes. Inject a dose of 0.2 ml of each mixture into guinea-pigs under the conditions described in the determination of the Lr/100 dose of the toxin.

The mixture of the preparation being examined that contains 0.01 Unit in 0.2 ml is the mixture that produces the same degree of local reaction as that produced by the injection into the same animals of the mixture of the Standard Preparation that contains in 0.2 ml the test dose (Lr/100) of the toxin and 0.01 Unit of antitoxin.

When at least four distinct tests are carried out by this method, the limits of error have been estimated to be 90 and 111 per cent.

2.15 BIOLOGICAL ASSAY OF GAS-GANGRENE ANTITOXIN (OEDEMATIENS)

The potency of gas-gangrene antitoxin (oedematiens) is determined by comparing the dose necessary to protect mice or other suitable animals against the toxic effect of gas-gangrene toxin (oedematiens) with the dose of the Standard Preparation of gas-gangrene antitoxin (*Clostridium oedematiens*) necessary to give the same protection. For this purpose the Standard Preparation of gas-gangrene antitoxin (*Clostridium oedematiens*) and a suitable preparation of gas-gangrene toxin (oedematiens), for use as a test toxin, are required. The test dose of the toxin is determined in relation to the Standard Preparation and the potency of the preparation being examined is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation and Unit

The Standard Preparation is a dried serum or any other suitable preparation of gas-gangrene antitoxin (oedematiens), the potency of which has been determined in relation to the International Standard.

The Unit is the specific neutralising activity for gas-gangrene toxin (oedematiens) contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

(1) Test toxin : Prepare gas-gangrene toxin (oedematiens) from a sterile filtrate of *Clostridium oedematiens*, the filtrate being prepared after about five days' growth of the organisms, by precipitation with *ammonium sulphate*; the resulting precipitate, which contains the toxin, is collected, dried *in vacuo* over *phosphorus pentoxide*, powdered and kept dry.

(2) Selection of test toxin : Select toxin for use as the test toxin by determining the following quantities:

L + dose – This is the smallest quantity of the toxin which, when mixed with 1 Unit of antitoxin and injected intramuscularly into mice, causes the death of the animals within seventy-two hours.

LD_{50} – This is the smallest quantity of toxin which, when injected intramuscularly into mice, causes the death within seventy-two hours of one half of the mice injected.

A suitable toxin is one which has an L+dose in 0.5 mg or less and contains not less than 25 LD_{50} in an L+dose.

(3) Determination of test dose of toxin : Prepare a solution of the Standard Preparation with *saline solution* so that 1 ml contains 10 Units. Weigh accurately a quantity of the dried toxin and dissolve it in *saline solution* so that 1 ml contains a precise amount, such as 10 mg.

Prepare mixtures so that 2 ml of each mixture contains 1 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the test toxin. Dilute each mixture with *saline solution* to the same final volume (2 ml).

Allow the mixtures to stand at room temperature for sixty minutes and, using six mice for each mixture, inject into each mouse, intramuscularly, a dose of 0.2 ml. Observe the mice thereafter for seventy-two hours.

If all the mice are killed, the amount of toxin present in 0.2 ml of the mixture is in excess of the test dose. If none of the mice is killed, the amount of toxin present in 0.2 ml of the mixture is less than the test dose. Prepare fresh mixtures such that 2 ml of each mixture contains 1 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the toxin intermediate between the smallest volume which killed all the mice and the largest volume which failed to kill any of the mice. Dilute each mixture to the same final volume (2 ml) with *saline solution*. Allow the mixtures to stand

for sixty minutes. Inject a dose of 0.2 ml of each mixture, intramuscularly into each of six mice. Observe the mice thereafter for seventy-two hours. Repeat the determination and add together the results of the separate tests which have been made with mixtures of the same composition so that a series of totals is obtained, each total representing the mortality due to one mixture.

The test dose of toxin is the amount present in 0.2 ml of that mixture which causes the death of one half of the total number of mice injected with it.

(4) Determination of the potency of the antitoxin:

Preliminary test – Weigh accurately a quantity of the test toxin and dissolve it in *saline solution* so that 1 ml contains ten times the test dose.

Prepare mixtures such that 2 ml of each mixture contains 1 ml of the solution of the toxin and one of a series of graded volumes of the preparation being examined. Dilute each mixture with *saline solution* to the same final volume (2 ml). Allow the mixtures to stand for sixty minutes. Inject a dose of 0.2 ml of each mixture into each of three mice under the conditions described for the determination of the test dose of toxin. If none of the mice is killed, 0.2 ml of the mixture contains more than 1 Unit of antitoxin. If all the mice are killed, 0.2 ml of the mixture contains less than 1 Unit of antitoxin.

Final test – Prepare similar fresh mixtures such that 2 ml of each mixture contains 1 ml of the solution of the test toxin and amounts of the preparation being examined intermediate between the smallest volume which protects all of the mice and the largest volume which fails to protect any of the mice, as determined by the preliminary test. Prepare a further mixture such that 2 ml contains 1 ml of the solution of the toxin and 10 Units of antitoxin. Dilute each mixture with *saline solution* to the same final volume (2 ml).

Allow the mixtures to stand for sixty minutes. Inject a dose of 0.2 ml of each mixture into each of six mice under the conditions described in the determination of the test dose of toxin.

The mixture of the preparation being examined that contains 1 Unit in 0.2 ml is that mixture which, killing some but not all of the mice, kills the same, or most nearly the same, number as the reference mixture containing 1 Unit of antitoxin in 0.2 ml.

Limits of error – The limits of error (P=0.99) attainable with the test are : Between 78 and 130 per cent when two animals per dose are used, between 83 and 120 per cent when four animals per dose are used, and between 86 and 116 per cent when six animals per dose are used.

2.16 BIOLOGICAL ASSAY OF GAS-GANGRENE ANTITOXIN (PERFRINGENS)

The potency of gas-gangrene antitoxin (perfringens) is determined by comparing the dose necessary to protect mice or other suitable animals against toxic effect of gas-gangrene toxin (perfringens, type A) with the dose of the Standard Preparation of gas-gangrene antitoxin (perfringens) (*Clostridium welchii* type A antitoxin) necessary to give the same protection. For this comparison the Standard Preparation of gas-gangrene antitoxin (perfringens) and a suitable preparation of gas-gangrene toxin (perfringens, type A) for use as a test toxin, are required. The test dose of the toxin is determined in relation to the Standard Preparation and the potency of the preparation being examined is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation and Unit

The Standard Preparation is a dried serum or any other suitable preparation of gas-gangrene antitoxin (perfringens), the potency of which has been determined in relation to the International Standard.

The Unit is the specific neutralising activity for gas-gangrene toxin (perfringens) contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

(1) Test toxin : Prepare gas-gangrene toxin (perfringens) from a sterile filtrate of a young culture of *Clostridium perfringens*, type A, by precipitation with *ammonium sulphate*; the resulting precipitate, which contains the toxin, is collected, dried *in vacuo* over *phosphorus pentoxide*, powdered and kept dry.

(2) Selection of test toxin : Select toxin for use as the test toxin by determining the following quantity:

L+ dose – This is the smallest quantity of the toxin which, when mixed with 1 Unit of antitoxin and injected intravenously into mice, causes the death of the animals within two days.

A suitable toxin is one which has an L+ dose of 4 mg or less, and one-twentieth or less of which is lethal for mice.

(3) Determination of test dose of toxin : Prepare a solution of the Standard Preparation in a *saline solution* such that 1 ml contains 5 Units. Weigh accurately a quantity of the dried toxin and dissolve it in *saline solution* so that 1 ml contains a precise amount, such as 10 mg.

Prepare mixtures such that 5 ml of each mixture contains 2 ml of the solution of the Standard Preparation (10 Units) and one of a graded series of volumes of the solution of the toxin. Dilute each mixture with *saline solution* to the same final volume (5 ml).

Allow the mixtures to stand at room temperature for sixty minutes, inject a dose of 0.5 ml of each mixture into a tail vein of each of six mice, and observe the mice thereafter for seventy-two hours.

The test dose of toxin is the amount present in 0.5 ml of that mixture which causes the death of some of the mice but not all of them provided that mixtures containing larger amounts of toxin cause the death of all the mice injected and that mixtures containing smaller amounts of the toxin fail to kill any of the mice injected.

(4) Determination of the potency of the antitoxin:

Preliminary test – Weigh accurately a quantity of the test toxin and dissolve it in a saline solution so that 2 ml contain ten times the test dose.

Prepare mixtures such that 5 ml of each mixture contains 2 ml of the solution of the toxin and one of a series of graded volumes of the preparation being examined. Dilute each mixture with a *saline solution* to the same final volume (5 ml). Allow the mixtures to stand for sixty minutes. Inject a dose of 0.5 ml of each mixture into each of three mice under the conditions described in the determination of the test dose of toxin. If none of the three mice is killed, 0.5 ml of the mixture contains more than 1 Unit of antitoxin. If all three mice are killed, 0.5 ml of the mixture contains less than 1 Unit of antitoxin.

Final test – Prepare fresh mixtures such that 5 ml of each mixture contains 2 ml of the solution of the test toxin and one of a series of graded volumes of the preparation being examined intermediate between the volume of antitoxin which fails to protect any of the mice, as determined by the preliminary test. Prepare a further mixture such that 5 ml contains 2 ml of the solution of the toxin and 10 Units of antitoxin.

Allow the mixtures to stand for sixty minutes and inject a dose of 0.5 ml of each mixture into each of six mice under the conditions described in the determination of the test dose of toxin.

The mixture of the antitoxin being examined that contains 1 Unit in 0.5 ml is the mixture that, killing some but not all of the mice, kills the same, or most nearly the same number as the mixture of the Standard Preparation that contains 1 Unit of antitoxin in 0.5 ml.

Limits of error – The limits or error (P = 0.99) attainable with this test are : between 82.5 and 121 per cent when two animals per dose are used, between 87.5 and 114 per cent when four animals per dose are used, and between 89.5 and 112 per cent when six animals per dose are used.

2.17 BIOLOGICAL ASSAY OF GAS-GANGRENE ANTITOXIN(SEPTICUM)

The potency of the gas-gangrene antitoxin (septicum) is determined by comparing the dose necessary to protect mice or other suitable animals against the toxic effect of gas-gangrene toxin (septicum) with the dose of the Standard Preparation of gas-gangrene antitoxin (*Clostridium septicum*) necessary to give the same protection. For this purpose the Standard Preparation of gas-gangrene antitoxin *(Clostridium septicum)* and a suitable preparation of gas-gangrene toxin (septicum), for use as a test toxin, are required. The test dose of the toxin is determined in relation to the Standard Preparation and the potency of the preparation being examined is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation and Unit

The Standard Preparation is a dried dilution of serum of gas-gangrene antitoxin (septicum) in phosphate-buffered saline or any other suitable preparation the potency of which has been determined in relation to the International Standard.

The Unit is the specific neutralising activity for gas-gangrene (septicum) contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

(1) Test toxin : Prepare gas-gangrene toxin (septicum) from a sterile filtrate of a one to three days' culture of *Clostridium septicum* (vibrion septique) by precipitation with *ammonium sulphate*; the resulting precipitate, which contains the toxin, is collected, dried *in vacuo* over *phosphorus pentoxide*, powdered and kept dry.

(2) Selection of test toxin : Select toxin for use as the test toxin by determining the following quantities :

L+ dose – This is the smallest quantity of the toxin which, when mixed with 1 Unit of antitoxin and injected intravenously into mice, causes the death of the animals within seventy-two hours.

LD_{50} – This is the smallest quantity of toxin which, when injected intravenously into mice, causes the death within seventy-two hours of one half of the mice injected.

A suitable toxin is one which has an L + dose of 5.0 mg or less and contains not less than 25 LD_{50} in an L + dose

(3) Determination of test dose of toxin : Prepare a solution of the Standard Preparation in a saline solution so that 1 ml contains 5 Units. Weigh accurately a quantity of the dried toxin and dissolve it in saline solution so that 1 ml contains a precise amount, such as 20 mg.

Prepare mixtures such that 5 ml of each mixture contains 2 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the toxin. Dilute each mixture with a saline solution to the same final volume (5 ml).

Allow the mixtures to stand at room temperature for sixty minutes, inject a dose of 0.5 ml of each mixture into a tail vein of each of six mice, and observe the mice thereafter for seventy-two hours.

The test dose of toxin is the amount present in 0.5 ml of

that mixture which causes the death of some of the mice but not all of them, provided that mixtures containing larger amounts of toxin cause the death of all the mice injected and that mixtures containing smaller amounts of the toxin fail to kill any of the mice injected.

(4) Determination of the potency of the antitoxin :

Preliminary test – Weigh accurately a quantity of the test toxin and dissolve it in *saline solution* so that 2 ml contains ten times the test dose.

Prepare mixtures such that 5 ml of each mixture contains 2 ml of the solution of the toxin and one of a series of graded volumes of the preparation being examined. Dilute each mixture with *saline solution* to the same final volume (5 ml). Allow the mixtures to stand for sixty minutes. Inject a dose of 0.5 ml of each mixture into each of three mice under the conditions described in the determination of the test dose of toxin. If none of the three mice is killed, 0.5 ml of the mixture contains more than 1 Unit of antitoxin. If all three mice are killed, 0.5 ml of the mixture contains less than 1 Unit of antitoxin.

Final test – Prepare fresh mixtures such that 5 ml of each mixture contains 2 ml of the solution of the test toxin and one of a series of graded volumes of the preparation being examined intermediate between the volume of antitoxin which protects all the mice and the volume of antitoxin which fails to protect any of the mice, as determined by the Preliminary test. Prepare a further mixture such that 5 ml contains 2 ml of the solution of the toxin and 10 Units of antitoxin.

Allow the mixtures to stand for sixty minutes and inject a dose of 0.5 ml of each mixture into each of six mice under the conditions described in the determination of the test dose of toxin.

The mixture of the antitoxin being examined that contains 1 Unit in 0.5 ml, is the mixture that, killing some but not all of the mice, kills the same, or most nearly the same number as the mixture of the Standard Preparation that contains 1 Unit of antitoxin in 0.5 ml.

Limits of error – The limits of error (P = 0.99) attainable with this test are: between 83 and 120 per cent when two animals per dose are used, between 88 and 113.5 per cent when four animals per dose are used, and between 90 and 111 per cent when six animals per dose are used.

2.18 BIOLOGICAL ASSAY OF RABIES ANTISERUM

The potency of rabies antiserum is determined by comparing the dose necessary to protect mice against a lethal intracerebral dose of rabies virus with the dose of the Standard Preparation of anti-rabies serum necessary to give the same protection.

Standard Preparation

The Standard Preparation is a dried serum or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

Method

(1) Test animals : Use young, healthy adult mice, weighing between 10 and 14 g, of either sex, but of the same sex in any given test.

(2) Test virus : Any suitable strain of rabies virus of known potency, such as the CVS strain may be used.

(3) Test dose of virus : The test dose of virus is such that each mouse receives by intracerebral injection between 20 and 1000 LD_{50}, preferably about 100 LD_{50}. To determine the number of LD_{50} of virus used, mix the virus dilution used in the test with an equal quantity of a 2 per cent v/v solution of normal horse serum and maintain the mixture at 37° for one hour. Prepare ten-fold dilutions and inject them into mice. Carry out this test at the same time as the test for determining the potency of the anti-rabies serum. If the test does not show that the quantity of virus used lies between 20 and 1000 LD_{50} discard the result and repeat the test.

(4) Determination of the potency of the anti-rabies serum : Prepare a series of six two-fold dilutions of the Standard Preparation and of the preparation being examined using *water* containing 2 per cent v/v of normal horse serum. To each dilution add a quantity of a suspension of the test virus containing the test dose and keep the mixtures at 37° for one hour. Inject 0.03 ml of each mixture, intracerebrally, into not fewer than ten mice.

Observe the mice for two weeks after the injection. Mice dying before the fifth day after inoculation with virus are eliminated from the test; all the mice dying between the fifth and fourteenth days after showing signs of rabies are considered to have died of rabies; mice living at the fourteenth day but showing signs of rabies are counted as deaths from rabies. Calculate the result of the test by standard statistical methods. The preparation being examined passes the test if, in a single comparative assay, it is found to have 80 or more Units per ml. If it is found to have less than 80 Units per ml, two more similar assays may be carried out; the preparation passes the test if, in both these additional assays, it is found to have 80 or more Units per ml.

2.19 BIOLOGICAL ASSAY OF TETANUS ANTITOXIN

The potency of tetanus antitoxin is determined by comparing the dose necessary to protect mice against the paralytic effects of a fixed dose of tetanus toxin with the dose of the Standard Preparation of tetanus antitoxin necessary to give the same protection. For this comparison the Standard Preparation of tetanus antitoxin and a suitable preparation of toxin, for use as a test toxin, are required. The test dose of the toxin is determined in relation to the Standard Preparation and the potency of the preparation being

examined is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation and Unit

The Standard Preparation is a quantity of dried tetanus antitoxin. The Unit is the specific neutralising activity for tetanus toxin contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

(1) Test toxin : Prepare tetanus toxin from a sterile filtrate of an eight to ten days culture of *Clostridium tetani*. Test toxins may be prepared by adding this filtrate to *glycerin* in the proportion of 1 volume of the filtrate to 1 to 2 volumes of *glycerin*. This solution of test toxin is stored at or below 0° Test toxins may also be prepared in stable form by saturating the filtrate with *ammonium sulphate*, collecting the resulting precipitate, drying it over *phosphorus pentoxide*, and reducing it to a fine powder. The powder so obtained is preserved in the dry condition at a low temperature, either in sealed ampoules, or in vacuo over *phosphorus pentoxide*.

(2) Determination of test dose of toxin : First determine the Lp/10 (Limes paralyticum/10) dose of the test toxin. This is the smallest quantity of the toxin which when mixed with 0.1 Unit of the Standard Preparation and injected subcutaneously into mice causes tetanic paralysis within four days. The severity of the tetanic paralysis to be regarded as the end-point is such that the paralysis is readily recognised but not sufficiently extensive to cause significant suffering. For humane reasons the animals should be examined at least twice a day and should be killed as soon as the end-point is reached. Prepare a solution of the Standard Preparation in a suitable liquid such that 1 ml contains 0.5 Unit. Accurately measure or weigh a quantity of the test toxin and dilute it with, or dissolve it in, a suitable liquid. Prepare mixtures such that each contains 2 ml of the solution of the Standard Preparation (1 Unit), one of a series of graded volumes of the solution of the toxin, and sufficient of a suitable liquid to give a final volume of 5 ml. Allow the mixtures to stand at room temperature, protected from light, for at least one hour. After some time, inject a dose of 0.5 ml of each mixture subcutaneously into mice, five mice being used for each mixture, and observe the mice for four days.

The Lp/10 dose of the toxin is the amount present in 0.5 ml of that mixture that contains the smallest amount of toxin sufficient to cause tetanic paralysis in all five mice injected with it within four days. All mice injected with mixtures containing larger amounts of toxin should develop paralysis earlier in the observation period; all those injected with mixtures containing smaller amounts should be without paralysis. If in a test all the mice injected with one mixture become paralysed early in the observation period and all the mice injected with the mixture containing the next smaller quantity of toxin are without paralysis, the test dose is intermediate between the quantities of toxin in 0.5 ml of each of the mixtures. If this is so, repeat the determination using fresh mixtures containing quantities of the test toxin including the expected end-point.

(3) Determination of the potency of the antitoxin : Prepare a solution of the Standard Preparation in a suitable liquid such that it contains 0.5 Unit per ml. Accurately measure or weigh a quantity of the test toxin and dilute it with, or dissolve it in, a suitable liquid so that 1 ml contains five times the Lp/10 dose as previously determined. Prepare mixture such that each contains 2 ml of the solution of the test toxin, one of a series of graded volumes of the preparation being examined, and sufficient of a suitable liquid to give a final volume of 5 ml. Prepare similar mixtures containing 2 ml of the solution of the test toxin and one of a series of graded volumes of the solution of the Standard Preparation centred on that volume (2 ml) that contains 1 Unit. Allow the mixtures to stand at room temperature, protected from light, for at least one hour. After this time, inoculate 0.5 ml of each mixture subcutaneously into mice, six mice being used for each mixture, and observe the mice for six days.

The mixture that contains the largest volume of the preparation being examined that fails to protect the mice from paralysis contains 1 Unit. The test is valid only if all the mice injected with mixtures containing 2 ml or less of the solution of the Standard Preparation show paralysis and all those injected with more, do not. Calculate the potency of the preparation being examined in Units per ml.

2.20 DETERMINATION OF THE Lf OF DIPHTHERIA VACCINE AND TETANUS VACCINE

The Limes flocculation is (Lf) of diphtheria vaccine or tetanus vaccine is determined by incubating together the preparation being examined and the Standard Preparation of diphtheria antitoxin or tetanus antitoxin for flocculation test in appropriate concentrations. When the concentration of the toxoid is kept constant and the concentration of the antitoxin varied in mixtures of constant volume, the mixture flocculating first is that which contains the most nearly equivalent quantities of toxoid and antitoxin.

Standard Preparation

The Standard Preparation is a freeze-dried serum of the relevant antitoxin or any other suitable preparation, the activity of which has been determined in relation to the International Reference Preparation.

Method

Carry out preliminary tests to determine the range of con-

centrations to be used. To each of a series of small tubes containing equal volumes of graded concentrations of the *Standard Preparation* add a constant volume of a solution of the preparation being examined. In successive tubes the antitoxin concentration should increase by amounts not greater than one-tenth of the concentration in the middle of the range of concentrations. Choose a range in such a manner that the optimum flocculating mixture will occur in the middle of the range. Heat the tubes in a water-bath at 45° to 50° with half the fluid column immersed to obtain convection currents. Continue heating and observe the tubes until the most rapidly flocculating mixture has been determined. The number of Lf in the quantity of the preparation being examined is numerically equal to the number of Lf equivalents of antitoxin in this mixture.

Limits of error – The error of a single determination has been estimated to be not more than 5 per cent.

2.21 BIOLOGICAL ASSAY OF ADSORBED DIPHTHERIA VACCINE

The potency of adsorbed diphtheria vaccine is determined by comparing the dose necessary to protect guinea-pigs either against the erythrogenic effects of an intradermal injection of diphtheria toxin or against the lethal effects of a subcutaneous injection of diphtheria toxin with the dose of the Standard Preparation of adsorbed diphtheria toxoid necessary to give the same protection. For this comparison, the Standard Preparation of adsorbed diphtheria toxoid and a suitable preparation of the diphtheria toxin, for use as a challenge toxin, are required.

Standard Preparation

The Standard Preparation consists of the toxoid adsorbed on aluminium hydroxide with serum, or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

Suggested Method

(a) INTRADERMAL CHALLENGE METHOD

Test animals : Use healthy, white or light-coloured guinea-pigs from the same stock, weighing between 250 and 350 g. Distribute them into six groups of sixteen and one group of four. The guinea-pigs should all be of the same sex or the males and females should be distributed equally between the groups.

Challenge toxin : Select a preparation of diphtheria toxin containing such an amount of free toxin that when it is diluted to contain 0.00025 Limes flocculation is (Lf) per 1.0 ml, a volume of 0.2 ml (0.00005 Lf) injected intradermally in the depilated flank of a guinea-pig causes a marked erythematous reaction visible forty-eight hours later

Preparation of the challenge toxin solutions : Immediately prior to use, prepare from the challenge toxin by dilution in *phosphate buffered saline pH 7.4* a challenge toxin solution containing 0.005 Lf in each 0.2 ml. Dilute a portion of this challenge toxin solution 1/100 in the same buffer solution.

Determination of potency : Prepare in *saline solution* three dilutions of the vaccine being examined and three dilutions of the Standard Preparation such that for each, the dilutions form a series differing by 2.5-fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1.0-ml volumes into guinea-pigs, protect approximately 50 per cent of the animals from the erythrogenic effects of the intradermal injection of the quantity of diphtheria toxin prescribed for this test. Allocate the six dilutions, one to each of the six groups of sixteen guinea-pigs, and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the group to which that dilution is allocated. After twenty-eight days shave one flank of each guinea-pig and inject each animal intradermally with 0.2 ml of the challenge toxin solution (0.005 Lf). Inject the guinea-pigs in the group of four similarly with 0.2 ml of the challenge toxin solution and also at adjacent sites, with 0.2 ml of the challenge toxin. Examine the guinea-pigs two days later for erythematous reactions to the toxin and calculate the potency of the vaccine being examined relative to the potency of the Standard Preparation on the basis of the numbers of animals that exhibit significant erythematous reactions in each of the six groups of sixteen, using the usual statistical methods. The test is valid only if for both the vaccine being examined and the Standard Preparation the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the guinea-pigs, the four guinea-pigs injected intradermally with challenge toxin and with the 1/100 dilution of the challenge toxin show erythematous reactions at the sites of both injections, and the statistical analysis shows a significant slope of the log dose-response lines, without significant deviations from either linearity or parallelism.

The vaccine passes the test if the lower fiducial limit of error of the estimated potency is not less than 30 Units of diphtheria toxoid potency per dose. The test may be repeated any number of times but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

(b) LETHAL CHALLENGE METHOD

Test animals : Use healthy, white or light-coloured guinea-pigs from the same stock, weighing between 250 and 350 g. Distribute them into six groups of sixteen and four groups of five. The guinea-pigs should all be of the same sex or the males and females should be distributed equally between the six groups of sixteen.

Challenge toxin : Select a preparation of diphtheria toxin containing not less than 100 LD_{50} in 1.0 ml.

Preparation of the challenge toxin solutions : Immediately prior to use, prepare from the challenge toxin by dilution in *phosphate buffered saline pH 7.4*, a challenge toxin solution containing approximately 100 LD_{50} in 1.0 ml. Dilute portions of this challenge toxin solution 1/32, 1/100, and 1/320 in the same buffer solution.

Determination of potency : Prepare in *saline solution* three dilutions of the vaccine being examined and three dilutions of the Standard Preparation such that for each, the dilutions form a series differing by 2.5-fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1.0 ml volumes into guinea-pigs, protect approximately 50 per cent of the animals from the lethal effects of the subcutaneous injection of the quantity of diphtheria toxin prescribed for this test. Allocate the six dilutions, one to each of the six groups of sixteen guinea-pigs, and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the groups to which that dilution is allocated. After twenty-eight days inject subcutaneously into each animal in the six groups of sixteen, 1.0 ml of the challenge toxin solution. Allocate the challenge toxin solution and the three dilutions made from it, one to each of the four groups of five guinea-pigs and inject subcutaneously 1.0 ml of each toxin solution into each guinea-pig in the group to which that solution is allocated. Count the number of surviving animals four days later and calculate the potency of the vaccine being examined relative to the potency of the Standard Preparation on the basis of the number of animals that survive in each of the six groups of sixteen, using the usual statistical methods. The test is valid only if for the vaccine being examined and the Standard Preparation the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the guinea-pigs, the survivors among the four groups of five guinea-pigs injected with the challenge toxin and its dilutions indicate that the challenge was approximately 100 LD_{50} and if the statistical analysis shows parallelism, linearity and a significant slope of the dose-response lines.

The vaccine passes the test if the lower fiducial limit of error of the estimated potency is not less than 30 Units of diphtheria toxoid potency per dose. The test may be repeated any number of times but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

2.22 BIOLOGICAL ASSAY OF PERTUSSIS VACCINE

The potency of pertussis vaccine is determined by comparing the dose necessary to protect mice against a lethal intracerebral dose of *Bordetella pertussis* with the dose of the Standard Preparation of pertussis vaccine necessary to give the same protection.

Standard Preparation and Unit

The Standard Preparation is a freeze-dried killed suspension of *Bordetella pertussis* or any other suitable preparation, the potency of which has been determined in relation to the Standard Reference Preparation from the Central Research Institute, Kasauli.

The Unit is the specific immunising activity contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Method

Distribute healthy white mice weighing between 13 and 15 g and drawn from a uniform stock into six groups of sixteen and four groups of ten; the mice should be of the same sex or the sexes should be equally distributed among the groups. Three of the groups of sixteen receive the Standard Preparation and the other three the vaccine being tested; the four groups of ten are used for the titration of the LD_{50} of the challenge suspension.

Use three doses of the Standard Preparation, suspended in *sodium chloride injection*, and three of the vaccine being tested, diluted with *sodium chloride injection*. In each case, the three doses should be so arranged that the dose protecting 50 per cent of the mice (ED_{50}) is as near as possible to the middle of the dose range. Suggested doses are 0.5, 0.1, and 0.02 Units of the Standard Preparation and 0.5 ml of a 1 in 8, a 1 in 40, and a 1 in 200 dilution of the vaccine. Give each mouse one dose, by intraperitoneal injection.

Fourteen to seventeen days later, challenge the mice by giving an intracerebral injection of 0.03 ml of a suspension of *B. pertussis* equivalent to approximately 1/6000 of the standard opacity. Prepare the suspension from a twenty-four hour growth on a suitable solid medium of a smooth culture of *B.pertussis* using a 1 per cent aqueous solution of casein hydrolysate containing 0.23 per cent *sodium chloride* and adjusted to pH 7.1 ± 0.1. At the same time, inject the four groups of ten mice with suitable dilutions of the suspension to determine the number of LD_{50} in the dose given to the inoculated mice. The challenge suspension should contain approximately 100 to 500 LD_{50} for non-immunised white mice. Observe the mice daily for fourteen days after the injection of the suspension of *B. pertussis*.

Calculate the results of the assay by standard statistical methods from the number of deaths, ignoring those which occur in the first two days. Repeat the test if necessary to ensure that the lower fiducial limit of error of the estimated potency is not less than 2 Units per dose.

The test is valid if (1) the ED_{50} of the vaccine being examined and of the Standard Preparation lies between the larger and the smaller vaccinating doses; (2) the protective response is graded in relation to the vaccinating dose, and (3) if the total number of survivals is not less than two or not greater than six with the vaccine under test in comparison with the Standard Preparation.

2.23 BIOLOGICAL ASSAY OF PLAGUE VACCINE

The potency of plague vaccine is estimated by determining the dose necessary to protect mice against a lethal dose of a virulent strain of *Pasteurella pestis*.

Test Animals

Use white mice, six to seven weeks old, each weighing between 22 and 28 g, and of a strain susceptible to plague infection. The selected strain of mice should be such that an infective dose of 6 to 12 organisms of a virulent strain of *Pasteurella pestis* per mouse given subcutaneously produces a mortality of not less than 80 per cent of the animals used. The animals should be healthy and free from intercurrent infection with organisms such as *Salmonella*.

Method

(1) Selection of suitable virulent strain : Prepare primary cultures by plating venous blood from patients with severe septicaemia, on agar slopes and incubating at a temperature between 27° and 32° for four days. After testing the growth for purity, seed a 2-mm loophole into 10 ml of nutrient broth in a test-tube with an internal diameter of 17 mm and incubate at 28° for forty-eight hours. Subculture by inoculating 0.5 ml of the well-shaken culture into 9.5 ml of nutrient broth contained in another test-tube and incubate at 28° for exactly forty-eight hours. Such a culture should contain 300 to 600 million organisms per ml. Make ten-fold dilutions in nutrient broth and test for virulence. Use a 10^{-7} dilution containing 6 to 12 organisms in 0.2 ml as the test infective dose per animal. Those strains which produce a mortality of 80 per cent among the animals tested with this dose are considered to be virulent. Such strains may be subcultured in rabbit blood agar and then stored at a temperature between 2° and 6° or alternatively may be freeze-dried.

(2) Standard challenge dose : Freshly reconstitute the selected culture or freeze-dried culture and dilute with nutrient broth to a strength such that 0.2 ml contains 60 to 120 organisms.

(3) Measurement of protective power : Prepare a series of five graded doses of the preparation being examined, arranged in such a manner that the 50 per cent protective dose lies about the middle of the selected series. For each dose a batch of six mice is used. Inject subcutaneously the selected dose in two equal parts with an interval of seven days between them. Seven days after the second half of the dose, inject subcutaneously into each group of mice, the standard challenge dose. At the same time inject into ten control mice, eight to nine weeks old and weighing between 28 and 30 g, the standard challenge dose.

Observe the animals for fifteen days and record the number of deaths in each group. Carry out a post-mortem on the dead animals and look for signs of plague in them. If plague organisms are not seen, such deaths are excluded from the calculations. The test is invalid if the number of such deaths is large. At the end of the period of observation kill all the surviving animals and examine for signs of plague.

Calculate the effective immunising dose, ED_{50} by standard statistical methods. A vaccine passes the test if it has a median effective immunising dose of 0.004 ml or less per mouse.

2.24 BIOLOGICAL ASSAY OF RABIES VACCINE

The potency of rabies vaccine is determined by comparing the dose necessary to protect mice from the lethal effects of a dose of rabies virus, administered intracerebrally, with the dose of the Standard Preparation of rabies vaccine necessary to give the same protection.

Standard Preparation

The Standard Preparation is a freeze-dried preparation, the potency of which has been determined in relation to the International References Preparation. It may be obtained from the Central Research Institute, Kasauli.

Test Animals

Use white mice weighing between 11 g and 15 g and three to four weeks old. The selected strains should be such that 0.03 ml per animal, containing 5-10 LD_{50} standard challenge virus suspension will produce 100 per cent mortality.

Method

(1) Distribute the mice into six groups of sixteen (for the potency test) and four groups of ten (for titrating the challenge virus). Use mice of the same sex or if of different sexes, distribute the sexes equally between the groups. Throughout the test, count as failing to resist the challenge, all mice that die with signs of rabies between the fifth and fourteenth day after injecti n with virus and those that are alive but show signs of rabies on the fourteenth day after challenge, exclude from the test any mice that die before the fifth day after challenge.

(2) Standard challenge virus suspension : The challenge virus strain is available in the freeze-dried form from the Central Research Institute, Kasauli. A working pool of the challenge virus strain is prepared by injecting intracerebrally 0.03 ml of a 10^{-2} dilution of the standard strain in 2 per cent sterile inactivated normal horse serum in sterile *water*, into a suitable number of test animals. The animals when moribund after showing characteristic symptoms of encephalitis are sacrificed and their brains harvested aseptically. They are then washed in chilled *physiological saline* to remove blood clots. A 10 per cent suspension of the brains is made in 10 per cent acid-digested casein hydrolysate at pH 7.2 and thoroughly homogenised.

After centrifuging lightly the supernatant liquid is distributed into sterile ampoules and freeze-dried. The hermetically sealed, freeze-dried ampoules are stored at 4°. In the lyophilised state, the virus titre is maintained for at least three years. Alternatively, the washed brains are homogenised in *buffered glycerin solution pH 7.2* to give a 10 per cent suspension. It is then centrifuged lightly, distributed into sterile ampoules and hermetically sealed. The sealed ampoules are stored at −20°. In this state, the virus titre is retained for at least one year.

Determine the virus titre of the *standard challenge virus suspension* by carrying out the following test simultaneously with the test for potency of the vaccine:

(3) Virus titre of the challenge virus : Prepare three serial dilutions of the *standard challenge virus suspension*. Using the four groups of ten mice, inject 0.03 ml of the *standard challenge virus suspension*, or of one of the dilutions thereof, intracerebrally into each mouse, using a different group for each suspension. Observe the mice for fourteen days. Calculate the virus titre of the *standard challenge virus suspension* in LD_{50} per 0.03 ml by standard statistical methods from the number of mice in each group that survive the challenge.

(4) Determination of potency of the vaccine : Reconstitute the Standard Preparation with a suitable liquid. Prepare three five-fold serial dilutions of the solution of the Standard Preparation and three five-fold serial dilutions of the preparation being examined and allocate one dilution to each of the six groups of sixteen mice. Inject each mouse in each group, intraperitoneally, with 0.03 ml of the dilution allocated to the group. After seven days, prepare six similar dilutions and repeat the injections. For both the Standard Preparations and the preparation being examined the serial dilutions should be prepared in such a way that the lowest dilutions protect from challenge more than 50 per cent of the mice to which they are given and the highest dilutions protect from challenge less than 50 per cent of the mice to which they are given.

After a further seven days inject each vaccinated mouse intracerebrally with 0.03 ml of the *standard challenge virus suspension*. Observe the mice for fourteen days. Calculate the potency of the preparation being examined by standard statistical methods from the number of mice that survive the challenge. The test is valid only if, for both the preparation being examined and the Standard Preparation, the 50 per cent protective dose lies between the largest and smallest doses given to the mice, there are no significant deviations of the log dose-response curves either from parallelism or from linearity, and the virus titre of the *standard challenge virus suspension* is between 5 and 50 LD_{50}.

2.25 TEST FOR LIVING CONTAMINANTS IN SMALL POX VACCINE

Reconstitute the vaccine to the form in which it is to be used for human inoculation. Pool the contents of the containers selected; the total volume should be not less than 0.25 ml. Carry out the following tests on the vaccine collected in this manner.

A. Test for Bacillus anthracis

Spread 0.1 ml of the vaccine upon the surface of a suitable medium such as *soybean-casein digest agar medium* and incubate at 37° for forty-eight hours. No organisms having the cultural, morphological and pathological characteristics of *Bacillus anthracis* should be found. Organisms morphologically resembling *B. anthracis* should be further examined. If the organisms are non-mobile further tests for the cultural character of *B.anthracis* should be made, including tests for pathogenicity in suitable animals. The vaccine should be free from *B.anthracis*.

B. Test for Escherichia coli

Make a 1:100 dilution of the vaccine and inoculate 1-ml aliquots into a suitable medium or spread on the surface of a medium suitable for differentiating *E.coli* from other bacteria and incubate at 37° for forty-eight hours. The vaccine should be free from *E.coli*.

C. Test for Clostridium tetani and other Spore-bearing Anaerobes

Distribute 1 ml of the vaccine in equal amounts into ten tubes, each containing not less than 10 ml of a medium suitable for the growth of anaerobic micro-organisms such as *fluid thioglycollate medium*. Hold the tubes at 65° for one hour in order to reduce the content of non-spore forming organisms and then incubate at 37° for at least seven days. No organisms having the cultural, morphological and pathological characteristics of *C.tetani* should be present. If any organisms resembling *C.tetani* are found, test the tube showing such organisms, for pathogenicity by inoculation into test animals in the following manner:

Use groups of not less than two guinea-pigs and five mice for each tube culture to be tested. Mix 0.5 ml of the culture with 0.1 ml of a freshly prepared 4 per cent w/v solution of *calcium chloride* and inject intramuscularly into each of the guinea-pigs; mix 0.2 ml of the culture with 0.1 ml of the calcium chloride solution and inject intramuscularly into each of the mice. Observe the animals for a week. The animals should not develop symptoms of tetanus and no animal should die as a result of infection with spore-forming anaerobes.

D. Test for Haemolytic Streptococci and Coagulase-positive Staphylococci

Make a 1:100 dilution of the vaccine and spread 1 ml upon the surface of not less than three plates of blood agar. Incubate the plates at 37° for forty-eight hours and examine the colonies. No organisms having the cultural,

morphological and haemolytic characteristics of *B. haemolytic streptococci* should be found.

2.26 DETERMINATION OF POTENCY OF SMALL POX VACCINE

A. Potency Test in Rabbits

Make serial ten-fold dilutions of the reconstituted vaccine being examined and of the Reference Preparation of Smallpox Vaccine. Using the 1:1000 and 1:10,000 dilutions of the vaccine being examined and of the reference preparation apply 0.1 ml to an area of approximately 5 sq cm or 0.2 ml to an area of approximately 10 sq cm of skin on the back of flank of at least two healthy rabbits. After four to seven days confluent lesions characteristic of the vaccine should be seen in the area to which the 1:1000 dilutions of the vaccine under test and of the reference preparation were applied, and more than one characteristic discrete lesion should be seen in the area to which the 1:10,000 dilutions were applied.

B. Enumeration of Pack-forming Units

Divide into two equal groups at least ten chick embryos, each of about twelve days' incubation. To the choriallantoic membrane of each embryo of the first group apply 0.1 ml or 0.2 ml of a suitable dilution of the reconstituted vaccine being examined. To the membrane of each of the second group of embryos apply 0.1 ml or 0.2 ml of another suitable dilution of the reconstituted vaccine. After incubation for not less than forty-eight hours at 37° count the total number of discrete specific lesions on the membrane of each embryo. From the dilutions employed, compute the total number of pack-forming units in 1 ml of the undiluted, reconstituted vaccine. This number should be not less than 5×10^7.

2.27 BIOLOGICAL TEST FOR TETANUS ANTITOXIN

The potency of tetanus vaccine or the tetanus component of a mixed vaccine is determined by assessing the ability of the vaccine to stimulate the production of tetanus antitoxin in guinea-pigs to which the vaccine has been administered in the manner described in the individual monograph. The sera of the guinea-pigs are examined for antitoxin by comparing their ability to protect mice from the paralytic effects of a fixed dose of a suitable preparation of tetanus toxin with the ability of the Standard Preparation of tetanus antitoxin to give the same protection. The Standard Preparation is used to determine an appropriate dose of the test toxin which is subsequently used in tests of the neutralising properties of the serum being tested.

Standard Preparation

The Standard Preparation is a dried serum the potency of which may be indicated by the Ministry of Health & Family Welfare, Government of India, from time to time in terms of the quantity exactly equivalent to the Unit accepted for international use.

Method

(1) Preparation of test toxin : Prepare tetanus toxin from a sterile filtrate of an eight to ten days' culture of *Clostridium tetani*. Test toxin may be prepared by adding this filtrate to *glycerin* in the proportion of 1 volume of the filtrate to 1 or 2 volumes of *glycerin*. This solution of test toxin is stored at or below 0°. Test toxins may also be prepared in stable form by saturating the filtrate with *ammonium sulphate*, collecting the resulting precipitate, drying it over *phosphorus pentoxide*, and reducing it to a fine powder. The powder so obtained is preserved in the dry condition at a low temperature, either in sealed ampoules, or in vacuo over *phosphorus pentoxide*.

(2) Selection of test toxin : Determine the Lp/200 (Limes paralyticum/200) dose of the test toxin. This is the smallest quantity of the toxin which, when mixed with 0.005 Unit of the Standard Preparation and injected into mice, causes tetanus paralysis within four days. Prepare a solution of the Standard Preparation by dissolving the contents of one ampoule in 1 ml of *water for injection* and adding sufficient *saline solution* to produce a total volume of 23 ml. Dilute a portion of the solution 1 in 200 with *saline solution* so that each 0.5 ml contains 0.025 Unit of tetanus antitoxin. Accurately measure or weigh a quantity of the test toxin, and dilute with or dissolve in, saline solution. Prepare mixtures such that each contains 0.5 ml of the solution of the Standard Preparation (0.025 Unit), one of a series of graded volumes of the solution of the test toxin, and sufficient *saline solution* to give a total volume of 2.5 ml. Allow the mixtures to stand at room temperature, protected from light, for at least one hour. After this time inject 0.5 ml of each mixture subcutaneously into mice, four mice being used for each mixture, and observe the mice for four days.

The mixture that contains the smallest amount of toxin sufficient to cause tetanic paralysis within the four-day period after injection contains five times the Lp/200 dose of the test toxin.

(3) Determination of potency of antitoxin : From the serum obtained from each guinea-pig prepare two dilutions, the first containing 0.5 ml of undiluted serum and 1.0 ml of *saline solution* and the second 0.5 ml of a 1 in 10 dilution of guinea-pig serum and 1.0 ml of *saline solution*. Prepare a fresh 1 in 200 dilution of the Standard Preparation and use to make a series of further dilutions containing, respectively, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml and 0.7 ml and sufficient *saline solution* to give a final volume of 1.5 ml. Lastly, prepare a fresh solution of the test toxin such that 1.0 ml contains five times the Lp/200 dose as determined previously and add 1 ml of this solution to each dilution of the guinea-pig sera and of the Standard Preparation. Allow the mixtures so prepared to stand at

room temperature, protected from light, for at least one hour. After this time inject 0.5 ml of each mixture subcutaneously into mice, two or three mice being used for each mixture. Survival of the mice injected with mixtures made with undiluted serum to the fourth day after injection, either without paralysis or with paralysis comparable with that observed in the mice injected with the mixture considered in the determination of the dose of test toxin to contain five Lp/200 doses of toxin, indicates a concentration of antitoxin in the undiluted serum of not less than 0.05 Unit per ml. Survival of the mice injected with mixtures made with serum diluted 1 in 10 to the fourth day after injection, either without paralysis or with paralysis comparable with that observed in the mice injected with the mixture considered in the determination of the dose of test toxin to contain five Lp/200 doses of the test toxin, indicates a concentration of antitoxin in the undiluted serum of not less than 0.5 Unit per ml. The test is valied only if the mice injected with mixtures containing 0.5 ml of the Standard Preparation developed paralysis comparable with that observed in the mice Injected with the mixture considered in the determination of the dose of test toxin to contain five Lp/200 doses of toxin, the mice injected with mixtures containing 0.3 and 0.4 ml of the Standard Preparation develop paralysis earlier in the observation period and the mice injected with mixtures containing 0.6 and 0.7 ml of the Standard preparation do not develop paralysis. Record the number of sera containing more than 0.05 Unit per ml and the number containing more than 0.5 Unit per ml.

2.28 BIOLOGICAL ASSAY OF ADSORBED TETANUS VACCINE

The potency of adsorbed tetanus vaccine is determined by comparing the dose of the vaccine required to protect guinea-pigs or mice from the paralytic effect of a subcutaneous injection of tetanus toxin with the dose of the Standard Preparation needed to give the same protection. For this comparison the Standard Preparation of adsorbed tetanus toxoid and a suitable preparation of tetanus toxin for use as a challenge toxin are necessary.

Standard Preparation

The Standard Preparation consists of toxoid adsorbed on aluminium hydroxide with serum, or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

Suggested Methods

(a) TEST IN GUINEA-PIGS

Test animals : Use healthy guinea-pigs from the same stock, weighing between 250 and 350 g Distribute them into six groups of sixteen and four groups of five. The guinea-pigs should all be of the same sex or the males and females should be distributed equally between the six groups of sixteen.

Challenge toxin : Select a preparation of the tetanus toxin containing not less than fifty 50 per cent paralytic doses per 1.0 ml.

Preparation of the challenge toxin solutions : Immediately prior to use prepare from this toxin by dilution in *phosphate buffered saline pH 7.4* a challenge toxin solution containing fifty 50 per cent paralytic doses in each 1.0 ml. Dilute portions of this challenge toxin solution 1/16, 1/50 and 1/160 in the same buffer solution.

Determination of potency : Prepare in *saline solution* three dilutions of the vaccine being examined and three dilutions of a solution of the Standard Preparation such that, for each, the dilutions form a series differing by 2.5-fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1.0-ml volumes into guinea-pigs, protect approximately 50 per cent of the animals from the paralytic effects of the subcutaneous injection of the quantity of tetanus toxin prescribed for this test. Allocate the six dilutions one to each of the six groups of sixteen guinea-pigs and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the group to which that dilution is allocated. After twenty-eight days inject each animal in the six groups of sixteen subcutaneously with 1.0 ml of the challenge toxin solution containing fifty 50 per cent paralytic doses. Allocate the challenge toxin solution and the three dilutions made from it one to each of the four groups of five guinea-pigs and inject subcutaneously 1.0 ml of each toxin solution into each guinea-pig in the group to which that toxin solution is allocated. Count the number of guinea-pigs without paralysis five days later and calculate the potency of the vaccine being examined relative to the potency of the Standard Preparation on the basis of the numbers of animals without paralysis in each of the six groups of sixteen, using standard statistical methods. The test is valid only if, for both the vaccine being examined and the Standard Preparation, the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the guinea-pigs, the numbers of unparalysed animals among the four groups of five injected with the challenge toxin and its dilutions indicate that the challenge was approximately fifty 50 per cent paralytic doses and the statistical analysis shows a significant slope of the log dose-response lines, without significant deviations from either linearity or parallelism.

The vaccine passes the test if the lower fiducial limit of error of the estimated potency is not less than 40 Units of tetanus toxoid potency per dose. The test may be repeated any number of times, but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

(b) TEST IN MICE

Test animals : Use healthy mice from the same stock, weighing between 17 g and 22 g. Distribute them is six groups of sixteen and four groups of six. The mice should all be of the same sex or the males and females should be distributed equally between the six groups of sixteen.

Challenge toxin : Select a preparation of tetanus toxin containing not less than one hundred 50 per cent paralytic doses per 1.0 ml.

Preparation of the challenge toxin solutions : Immediately prior to use prepare from this toxin by dilution in *phosphate buffered saline pH 7.4* a challenge toxin solution containing fifty 50 per cent paralytic doses in each 0.5 ml. Dilute portions of this challenge toxin solution 1/16, 1/50, and 1/160 in the same buffer solution.

Determination of potency : Prepare in *saline solution* three dilutions of the vaccine being examined and three dilutions of a solution of the Standard Preparation such that, for each, the dilutions form a series differing by 2.5-fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 0.5 ml volumes into mice, protect approximately 50 per cent of the animals from the paralytic effects of the subcutaneous injection of the quantity of tetanus toxin prescribed for this test. Allocate the six dilutions one to each of the six groups of sixteen mice and inject subcutaneously 0.5 ml of each dilution into each mouse in the group to which the dilution is allocated. After twenty-eight days inject each animal in the six groups of sixteen subcutaneously with 0.5 ml of the challenge toxin solution containing fifty 50 per cent paralytic doses. Allocate the challenge toxin solution and the three dilutions made from it one to each of the four groups of six mice and inject subcutaneously 0.5 ml of each toxin solution into each mouse in the group to which that toxin solution is allocated. Count the number of mice without paralysis four days later and calculate the potency of the vaccine being examined relative to the potency of the Standard Preparation on the basis of the numbers of animals without paralysis in each of the six groups of sixteen, using standard statistical methods. The test is valid only if, for both the vaccine being examined and the Standard preparation, the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the mice, the numbers of unparalysed animals among the four groups of six injected with the challenge toxin and its dilutions indicate that the challenge was approximately fifty 50 per cent paralytic doses and the statistical analysis shows a significant slope of the log dose-response lines, without significant deviations from either linearity of parallelism.

The vaccine passes the test if the lower fiducial limit of error of the estimated potency is not less than 40 Units of tetanus toxoid potency per dose. The test may be repeated any number of times, but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

2.29 BIOLOGICAL ASSAY OF TYPHOID VACCINE

The potency of typhoid vaccine is determined by comparing the dose necessary to protect mice from the lethal effects of a dose of *Salmonella typhi* with the dose of the Standard Preparation needed to give the same protection.

Standard Preparation and Unit

The Standard Preparation is a quantity of freeze-dried preparation of typhoid vaccine or any other preparation, the potency of which has been determined in relation to the International Standard.

The Unit is the specific immunising activity contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Method

Distribute mice drawn from stock of a single strain, of the same sex or an equal number of each sex in each group and each weighing between 13 and 16 g. Use at least three groups consisting of not less than sixteen mice each, for each vaccine and at least four groups consisting of not less than ten mice each, for control; One group for the challenge dose and three groups for titrating the virulence of the challenge dose.

Prepare serial dilutions, no greater than five-fold, of the substance being examined and of the Standard Preparation in *saline solution*, such that the mid-dilution of each vaccine contains that amount of vaccine which will afford protection to approximately 50 per cent of the mice. Inject intra-peritoneally into each mouse of each group 0.5 ml of the appropriate dilutions.

Seven to fourteen days later, challenge the mice by giving an intraperitoneal injection of 0.5 ml of the challenge suspension prepared in the following manner. From a freeze-dried culture of strain Ty 2 of *S. typhi* sub-culture on a suitable agar medium for 6 hours at 36° ± 1° by seeding from a 16 to 20 hour culture grown at 36° ± 1° on a suitable agar medium. Harvest the bacteria and suspend uniformly in *saline solution*. Remove from the suspension agar particles and clumps of bacteria, adjust to an opacity of 10 units and dilute in *saline solution* by ten-fold increments. Put the suspensions into a sterile gastric mucin preparation. Prepare the challenge suspension from whichever bacterial dilution which provides about 1000 colony-forming Units for an 0.5 ml challenge dose. The virulence titration suspensions shall be 10^1, 10^2, and 10^3 dilutions respectively of the challenge suspension.

Inoculate each mouse by injecting intraperitoneally 0.5 ml of the challenge suspension. At the same time, inject intraperitoneally the four groups of control mice with a 0.5 ml dose of the challenge suspension and its three dilutions, respectively. Inject the challenge dose control mice last. The interval between removal of the

bacteria from the culture medium and the injection of the last mouse shall not exceed two and a half hours. Observe the mice daily for three days after injection and record the number of mice that survive at the end of the observation period. Calculate the results of the assay by standard statistical methods.

The test is valid provided, (1) the ED_{50} of the preparation being examined and the Standard Preparation is between the largest and smallest doses inoculated; (2) the limits of the standard deviation of the ED_{50} of each vaccine fall within the ranges of 61 per cent to 163 per cent; and (3) a graded protective response is obtained in relation to the vaccine dilutions.

If the test does not meet these criteria repeat it.

Calculate the protective unit value per ml of the vaccine under test in terms of the unit value of the Standard Preparation. The vaccine shall have a potency of 8 Units per ml. Variations in potency unit estimates are acceptable provided the estimate is not less than 5.0 Units per ml.

2.30 BIOLOGICAL ASSAY OF TYPHUS VACCINE

Inject subcutaneously 0.5 ml of the vaccine being examined, into each of ten or more healthy guinea-pigs, each weighing between 400 and 500 g. After seven days inject once again 0.5 ml of the vaccine into the same animals. Fourteen days after the second injection bleed the animals and pool aliquots of the sera. Test the pools against a toxic substance prepared from injected yolk sacs of living fertile eggs incubated for four to ten days after inoculation with murine or epidemic typhus rickettsiae. Harvest the yolk sac and stain a smear by the Macchiavello technique. Use only yolk sacs of living embryos showing 4 + or more rickettsiae. Determine the wet weight of the yolk sac, grind the yolk sac with a suitable grade of aluminium oxide and suspend in sterile milk (pH adjusted to 7.6) or in a mixture of one part of fresh egg yolk and 99 parts of *sodium chloride injection* using, for each g of the yolk sac, 10 ml of the vehicle. Centrifuge this suspension for five minutes at low speed, decant the supernatant liquid and distribute in ampoules for freezing. Dilute a small amount of this suspension 1 to 8, 1 to 16, 1 to 32, and 1 to 64 with *saline solution*. Inject intravenously doses of 0.5 ml of each dilution into eight white mice, each weighing between 11 and 15 g. Eighteen hours later, estimate the toxicity of the preparation as the LD_{50} per g of yolk sac, calculated from the numbers of mice killed by each dilution. Those toxic suspensions showing 160 or more LD_{50} per g of yolk sac are satisfactory for use in the neutralisation test. The remainder of the suspension may be sealed in ampoules and preserved for eighteen months in liquid nitrogen.

Prepare mixtures of the toxic substance and the sera being tested for the neutralisation test in mice and allow to stand for two hours before injection. Inject intravenously into mice, each weighing between 11 and 15 g, 0.5 ml containing 2 LD_{50} of the toxic substance together with serial 1 to 2 dilutions of serum. Keep the mice in an incubator at a temperature of 28° to 30° until the results of the test are recorded. Record the deaths at the end of twenty-four hours and express the results in terms of the highest dilution giving complete protection.

2.31 BIOLOGICAL ASSAY OF OLD TUBERCULIN

The potency of a sample of old tuberculin is determined by comparing the dose necessary to reveal delayed hypersensitivity in guinea-pigs or other animals hypersensitised with *Mycobacterium tuberculosis* with the dose of the Standard Preparation necessary to give the same effect.

Standard Preparation and Unit

The Standard Preparation consists of a concentrate of the culture filtrate of *M. tuberculosis* in a 50 per cent w/v solution of *glycerin*, or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

The Unit is the specific biological activity contained in such an amount of the Standard Preparation as the Ministry of Health & Family Welfare, Government of India, may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Method

Sensitise twelve guinea-pigs, weighing between 300 and 400 g, by the intramuscular injection of a suitable dose of killed or live, attenuated or non-attenuated *M. tuberculosis*. If Bacillus Calmette-Guerin Vaccine is used for sensitising the animals, a suitable dose is 2 mg moist weight of the constituent bacilli. Not less than three weeks later the guinea-pigs are fully sensitised. Shave the flanks to provide space for six reactions on each side, thus providing twelve injection sites per animal. Use three doses of the Standard Preparation and three doses of the preparation being examined, the highest dose being ten times as strong as the lowest. Dilute the preparations so that the lesions produced are not less than 8 and not more than 25 mm in diameter. Allocate the doses to the available sites in a random manner, but with the constraints that each dose is allocated to two sites on each animal and allocated twice to sites in each position. Inject each dose intracutaneously in the same volume (0.1 or 0.2 ml) at the sites to which it has been allocated. Measure the diameters of the lesions after twenty-four hours and calculate the result of the test using standard statistical methods on the basis that the lesion diameters are directly proportional to the logarithm of the concentration of the tuberculin.

Limits of error : The fiducial limits of error of the assay are not less than 75 per cent and not more than 130 per cent of the stated potency.

2.32 BIOLOGICAL ASSAY OF TUBERCULIN PURIFIED PROTEIN DERIVATIVE

The potency of tuberculin purified protein derivative is determined by comparing the dose necessary to reveal delayed hypersensitivity in guinea-pigs or other animals hypersensitised with *Mycobacterium tuberculosis, bovis* with the dose of the Standard Preparation necessary to give the same effect.

Standard Preparation

The Standard Preparation consists of a mammalian purified protein derivative with salts, or any other suitable preparation, the potency of which has been determined in relation to the International Standard.

Method

Proceed as described under *biological assay of old tuberculin*, Appendix 2.31.

2.33 BIOLOGICAL ASSAY OF SNAKE VENOM

Dissolve a quantity of the freeze-dried venom equivalent to 50 Mouse Units in 25 ml of *saline solution*. Inject 0.5 ml intravenously into each of ten mice weighing between 18 g and 20 g and observe the animals for twenty-four hours. Not less than three and not more than eight of the mice die in two to twenty-four hours. If the number of deaths is not within this range, dilute the venom suitably.

NOTE – The quantity in mg of venom which will kill in two to twenty-four hours not less than three and not more than eight mice will represent one Mouse Unit.

2.34 TEST FOR URINARY EXCRETION OF DEXTRANS

Place two rabbits in separate metabolism cages and collect the urine of each for forty-eight hours. Into the marginal ear vein of each rabbit inject over a period of five to ten minutes a dose of 20 ml per kg of body weight of the preparation being tested and collect the urine excreted in the first forty-eight hours after injection. All the urine should be collected in clean glass vessels under *toluene*. Measure the total volume excreted by each of the rabbits and filter the urine.

Dialyse through a suitable membrane 10 ml of each sample of urine against running water for twenty-four hours and against *water* for further twenty-four hours. Transfer to a 25-ml graduated flask by means of a pasteur pipette, rinse the membrane with 6 to 8 ml of *water* and dilute the combined urine and rinsings to 25 ml with *water*. To 3.0 ml of this solution, or a suitable volume diluted to 3.0 ml with *water* in a test-tube cooled in water, add carefully 6.0 ml of a 0.2 per cent w/v solution of *anthrone* in a mixture of 19 volumes of *sulphuric acid* and one volume of *water*, so that the solutions form layers. Mix immediately the contents of the tube, place in a water-bath and heat for five minutes with precautions against loss of water by evaporation. Cool and compare the colour of the solution with that of a blank solution prepared in the same manner from 3.0 ml of *water*, using a colorimeter fitted with a red filter. Determine the apparent amount of dextrose in the solution from a reference curve prepared by treating 3.0 ml quantities of solutions of *dextrose anhydrous* by the same process (suitable amounts of dextrose for this purpose are 15, 30, 60 and 90 µg per 3.0 ml).

Calculate the percentage of dextrans excreted for each rabbit from the expression:

$$90\ (x-y)/w$$

where

x = the total apparent amount of dextrose, in mg, excreted in the first forty-eight hours after injection

y = the total apparent amount of dextrose, in mg, excreted in the forty-eight hours before injection

w = weight, in mg, of dextrans injected

The result of the test is determined on the mean of the results for the two rabbits and must not exceed 30 per cent.

2.35 TEST FOR HISTAMINE-LIKE SUBSTANCES

Reagents

(1) Heparinised saline solution : A sterile *saline solution* containing 50 Units of *heparin* in 1 ml.

(2) Standard histamine solution : Dissolve a suitable quantity of *histamine dihydrochloride* or *histamine acid phosphate* in sufficient *water* or *saline solution* to produce a solution containing 0.1 µg of histamine base, $C_5H_9N_3$ per ml.

Test Animal

Use a healthy, adult cat, either male or non-pregnant female weighing approximately 2.5 kg. Weigh the cat and anaesthetise it by intraperitoneal injection of an anaesthetic substance, such as chloralose or a suitable barbiturate, that allows the maintenance of uniform blood pressure. Immobilise the animal and protect it from loss of body heat. Maintain it so that the rectal temperature remains within physiological limits. Introduce a tube into the trachea. Expose a carotid or other suitable artery, separate it from surrounding tissues, insert a canula filled with *heparinised saline solution* and arrange for continuous blood-pressure recording with a manometer or other apparatus of equivalent sensitivity. Then expose a femoral vein to facilitate intravenous injection of solutions of histamine and of the substance being examined.

Determine the sensitivity of the animal to histamine by injecting intravenously, at regular intervals of not less than

five minutes, doses of *standard histamine solution* corresponding to 0.05, 0.1, and 0.15 µg of histamine base per kg of body weight of the animal. Repeat the injection of the dose of 0.1 µg per kg at least three times. Administer the second and subsequent injections not less than one minute after the blood pressure has returned to a constant level. Use the animal for the test only if the responses to the graded doses are clearly different and the response to the repeated injections of the dose of 0.1 µg per kg are approximately the same and correspond to a decrease in pressure of not less than 20 mm of mercury.

Method

Dissolve the substance being examined in sufficient *saline solution* or other diluent prescribed in the individual monograph, to give the test solution in the concentration specified in the monograph. Follow the same time schedule established during the injection of *standard histamine solution*. Inject intravenously per kg of the cat's weight, 1.0 ml of *standard histamine solution*, followed by an injection of the specified amount of the *test solution* and, finally, 1.0 ml of *standard histamine solution*. The second and third injections are given not less than one minute after the blood pressure has returned to a constant level. When a common canula is used for both the *standard histamine solutions* and *test solutions*, each injection of the standard and test solution should be immediately followed by an injection of approximately 2.0 ml of *saline solution* to flush any residues from the tubing. Measure the change in blood pressure following each of the three injections. The depressor response to the *test solution* is not greater than one-half the mean depressor response to the two associated doses of the *standard histamine solution*. If this requirement is not met, continue the series of injections similarly until it consists of five doses, of which the three doses of 1.0 ml each of *standard histamine solution* are alternated with two doses of the *test solution*. Measure the change in blood pressure following each of the additional injections. The substance passes the test if the depressor response to each dose of the *test solution* is not greater than the mean of the respective depressor responses to the associated doses of the *standard histamine solution*, representing 0.1 µg of histamine base per kg.

If the depressor response to either dose of the *test solution* is greater than the mean of the depressor response to the associated doses of the *standard histamine solution*, the test may be continued in the same animal, or in another animal similarly prepared and tested for responses to the *standard histamine solution*. If the test is continued in the same animal, after the last dose of the *standard histamine solution* of the initial series, administer four more injections, of which two are doses of the *test solution* and two are doses of 1.0 ml each, of *standard histamine solution* alternately in sequence. If the test is continued in another animal, prepare a fresh solution of the substance being examined for an independent container or containers of the substance and inject a series of five doses comprising the *standard histamine solution* and the *test solution* in accordance with the initial injection sequence. Measure the change in blood pressure following each of the additional injections. Compute the difference between each response to the dose of the *test solution* and the mean of the associated doses of the *standard histamine solution* in the entire series, initial and additional, and calculate the average of all such differences. The substance passes the test if the average of the differences is such that in the specified dose the depressor response to the *test solution* is not greater than the depressor response to the dose of the *standard histamine solution*, representing 0.1 µg of histamine base per kg and if not more than one half of the depressor responses to the *test solution* are greater than the mean of the respective depressor responses to the associated doses of the standard histamine solution, representing 0.1 µg histamine base per kg.

2.36 TEST FOR PYROGENS

The test involves the measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution of the substance being examined. It is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 ml per kg injected intravenously within a period of not more than ten minutes.

Recording of Temperature

Use an accurate temperature-sensing device such as a clinical thermometer or thermistor or other suitable probes that have been calibrated to assure an accuracy of ± 0.1° and have been tested to determine that a maximum reading is reached in less than five minutes. Insert the thermometer or temperature-sensing probe into the rectum of the test rabbit to a depth of not less than 7.5 cm, and, after a period of time not less than that previously determined as sufficient, record the rabbit's body temperature.

Materials

All glassware, syringes and needles must be thoroughly washed with *water for injection* and heated in a hot air oven at 250° for thirty minutes or at 200° for one hour. Treat all diluents and solutions for washing and rinsing of devices in a manner that will assure that they are sterile and pyrogen-free.

The retaining boxes for rabbits in which the temperature is being measured by an electrical device should be made in such a way that the animals are retained only by loosely-fitting neck-stocks; the rest of the body remains relatively free so that the rabbits may sit in a normal position. The animals must be put into the boxes one hour before the test and remain in them throughout the test.

Test Animals

Use healthy, adult rabbits of either sex, preferably of the same variety, weighing not less than 1.5 kg, fed on a com

plete and balanced diet and not showing loss of body weight during the week preceeding the test. House the animals individually in an area of uniform temperature (±2°), preferably with uniform humidity, and free from disturbances likely to excite them.

Do not use animals for pyrogen tests more frequently than once every 48 hours. After a pyrogen test in the course of which a rabbit's temperature has risen by 0.6° or more, or after a rabbit has been given a test substance that was adjudged pyrogenic, at least two weeks must be allowed to elapse before the animal is used again.

Preliminary Test (Sham Test)

If animals are used for the first time in a pyrogen test or have not been used during the two previous weeks, condition them one to three days before testing the substance being examined, by injecting intravenously into them 10 ml per kg of body weight of a pyrogen-free *saline solution*. Carry out the test in a room where there is no risk of disturbance of exciting the animals and in which the room temperature is within 3° of that of the area where the animals are housed or in which the animals have been kept for at least eighteen hours before the test. Withhold food from the animals overnight and until the test is completed; withhold water during the test.

Record the temperature of the animals, beginning at least ninety minutes before injection and continuing for three hours after injection of the solution being examined. Any animal showing a temperature variation of 0.6° or more must not be used in the main test.

Main Test

Carry out the test using a group of three rabbits.

Preparation of the sample : Dissolve the substance being examined in, or dilute with, a pyrogen-free *saline solution*. Warm the liquids being examined to approximately 38° before injection. The amount of the sample to be injected varies according to the preparation being examined and is prescribed in the individual monograph. The volume of injection is not less than 0.5 ml per kg and not more than 10 ml per kg of body weight.

Procedure : Record the temperature of each animal at intervals of not more than thirty minutes, beginning at least ninety minutes before the injection of the solution being examined and continuing for three hours after the injection. Not more than forty minutes immediately preceding the injection of the test dose, record the "initial temperature" of each rabbit, which is the mean of two temperature readings recorded for that rabbit at an interval of thirty minutes in the forty-minute period. In any one group of test animals, use only those animals whose "initial temperatures" do not vary by more than 1° from each other, and do not use any rabbit having a temperature higher than 39.8°.

Rabbits showing a temperature variation greatef than 0.2° between two successive readings in the determination of "initial temperature" should not be used for the test. Inject slowly the solution being examined into the marginal vein of the ear of each rabbit over a period not exceeding ten minutes, unless otherwise prescribed in the monograph. Record the temperature of each animal at half-hourly intervals for three hours after the injection. The difference between the "initial temperature" and the "maximum temperature" which is the highest temperature recorded for a rabbit is taken to be its response. When this difference is negative, the result is counted as a zero response.

Interpretation of results : If the sum of the responses of the group of three rabbits does not exceed 1.4° and if the response of any individual rabbit is less than 0.6°, the preparation being examined passes the test. If the response of any rabbit is 0.6° or more, or if the sum of the responses of the three rabbits exceeds 1.4°, continue the test using five other rabbits. If not more than three of the eight rabbits show individual responses of 0.6° or more, and if the sum of the responses of the group of eight rabbits does not exceed 3.7°, the preparation being examined passes the test.

2.37 TESTS FOR UNDUE TOXICITY

1. General Test

Inject intravenously into each of five healthy mice, weighing between 17 g and 22 g the quantity of the substance to be examined prescribed in the individual monograph, dissolved in 0.5 ml of *water for injection* or in *sodium chloride injection*. The time of injection should normally occupy about fifteen to thirty seconds, unless otherwise prescribed in the individual monograph.

The substances passes the test if none of the mice dies within twenty-four hours or within such time as is specified in the individual monograph. If one of the animals dies, repeat the test. The substance passes the test if none of the animals in the second group dies within the time interval specified.

2. For Vaccines and Sera

Unless otherwise prescribed in the individual monograph, inject intraperitoneally one human dose*, but not more than 1.0 ml, into each of five healthy mice, weighing between 17 g and 22 g, and one human dose, but not more than 5.0 ml, into each of the two healthy guinea-pigs, weighing between 250 g and 350 g.

The preparation passes the test if none of the animals shows signs of ill-health in the seven days following the injection. If one of the animals dies or shows signs of ill-health, repeat the test. The preparation passes the test if

* The human dose is that stated on the label of the preparation to be examined or in the accompanying information leaflet.

none of the animals in the second group dies or shows signs of ill-health in the time interval specified.

NOTE – During the test each group of animals administered a sample should be housed in a suitable container and receive adequate food and water. A constant temperature environment is desirable at all times.

2.38 TEST FOR MYCOBACTERIUM TUBERCULOSIS

(1) Centrifuge not less than 25 ml of the preparation to be examined, for one hour at a speed of about 1500 r.p.m. Suspend the sediment in 25 ml of a 0.9 per cent w/v solution of *sodium chloride*, or if an inhibiting substance has been used in the preparation, in 25 ml of a suitable neutralising solution. Centrifuge the suspension again for one hour and suspend the sediment in 15 ml of a 0.9 per cent w/v solution of *sodium chloride*.

Inoculate ten tubes each containing 25 ml of an appropriate liquid medium and ten tubes containing an appropriate solid medium with 0.5 ml of the suspension and incubate at 37° for forty-two days. The suitability of the medium must have been previously established by inoculating a suitable strain of *Mycobacterium tuberculosis* and observing the growth of the organism.

At the end of the incubation period, none of the tubes shows growth of mycobacteria; if growth occurs in any of the tubes, reject the batch. If during the first eight days of incubation contaminating microorganisms develop, repeat the test, carrying out at the same time a further *test for sterility*, Appendix 4.6.

(2) Inject 1 ml of the preparation to be examined, intramuscularly into each of five tubercular-negative guinea-pigs, each weighing between 250 g and 350 g. Observe the animals for sixty days. Not fewer than two must survive. Kill the survivors. All the animals when examined at autopsy are free from tubercular lesions.

2.39 DETERMINATION OF ABO GROUP AND Rh GROUP

To Determine the ABO Blood Group of Blood Donors

The blood is examined both for the agglutinogens of the red corpuscles and for agglutinins in the serum. A few ml of fresh blood, without anticoagulant, is allowed to clot in a narrow test tube, and the serum removed. The clot or a small part of it is stirred with a 0.9 per cent w/v solution of *sodium chloride*, the resulting suspension centrifuged and the corpuscles resuspended in a 0.9 per cent w/v solution of *sodium chloride* in *water* to make a 2 to 3 per cent v/v suspension of packed corpuscles.

Test for agglutinogens : The test is conveniently made in lipless tubes 50 mm × 6 mm, each containing 0.04 ml volumes of diagnostic serum, of the red corpuscles suspension, and of a 0.9 per cent w/v solution of *sodium chloride* (to avoid nonspecific agglutination) delivered from calibrated Pasteur pipettes. One tube contains anti-A serum, and a second contains anti-B serum.

It is advisable to control the specificity of the anti-A serum and the anti-B serum by testing them with red corpuscles of known groups A, B and O.

The tubes are shaken and left at room temperature for two hours, covered with cylindrical glass caps to prevent evaporation and to protect the contents. The caps are then removed and each tube flicked with a finger to disperse the deposit of corpuscles. The contents of tubes in which no obvious agglutination has occurred are examined microscopically at a magnification of ×50 to ×100, spread evenly on a slide with the stem of a Pasteur pipette. The reaction is scored as follows:

++++ Complete agglutination into one or two large clumps.

+++ Numerous clumps clearly visible to the naked eye.

++ Granularity just visible to the naked eye; very big clumps and unagglutinated corpuscles seen under the microscope.

\+ Not quite such big clumps; numerous unagglutinated corpuscles.

(+) Clumps of 8 to 12 corpuscles.

w A definite but weak reaction in which there is a uniform distribution of very small clumps of 4 to 6 corpuscles.

? Uneven distribution of corpuscles with no definite clumps.

– All corpuscles separate and evenly distributed.

In tests of red corpuscles a reaction less than + is unlikely to be due to A or B agglutinogens, and the corpuscles should be tested.

Test for agglutinins : The serum of the blood under examination is similarly tested against group A and B red corpuscles, 0.04 ml of suspension and 0.04 ml of undiluted serum being used. In this test, small reactions are frequent, and in about 50 per cent of infants under one year old, either one or both of the agglutinins to be expected on the basis of the agglutinogens of the red corpuscles, are not found. In all bloods, a "w" reaction is considered to be true, a "?" requires a re-test; and when the expected agglutinins are not disclosed after re-test, the diagnosis is based on the agglutinogens of the red corpuscles.

Slide or Tile method : Blood obtained from the finger-prick is diluted with normal physiological saline so as to give an approximate 5% R.B.C. suspension. Oxalated blood is also suitable for this purpose.

Two plain glass slides are marked A and B and one drop

of R.B.C. suspension is placed on each of them, add one drop of Anti-A Serum on the slide marked A and one drop of Anti-B Serum on the slide marked B. Mix with a glass rod and gently rock the slide to-and-fro. The agglutinate will appear almost at once with the appropriate serum.

Negative readings should not be taken until the preparation has stood at least for ten minutes. The reaction is visible to the naked eye but a hand lens or a low power microscope may be employed in doubtful cases. When large number of blood samples have to de grouped, a long porcelain or opal glass tile may be employed in place of glass slides.

Weak reactions with anti-A serum in group AB : Care must be taken in making the diagnosis of group B, because the reaction of anti-A serum with the A agglutinogen are often weak when the B agglutinogen is also present on the corpuscles; group AB corpuscles may therefore be falsely diagnosed as group B corpuscles. This is particularly true of the sub-group A, B, in which the reactions of the A agglutinogen are always weak. The examination of the serum for agglutinins does not necessarily serve as a check since the serum of a number of A, B persons contains the agglutinin anti-A, which reacts with A_1, but not with A_2 corpuscles. Since A_1 corpuscles are used to check the serum, agglutination may occur and the diagnosis of group B corpuscles be apparently confirmed. The mistake is however, unlikely to arise if an anti-A serum is used which has been tested for its capacity to react with known AB corpuscles. As an additional check the serum from every blood that has been shown by determination of its agglutinogen and agglutinin content to belong to group B is tested against known A_2 corpuscles. If there is a reaction it is clear that the serum must contain the agglutinin alpha, which reacts with A_2 as well as with A_1 corpuscles. The serum, therefore, cannot have come from an A_1B or an A_2B person and must have come from a group B person. The diagnosis that corpuscles belong to group B should not be made unless the serum reacts with A_2 corpuscles.

To Determine the Rh Group of Human Blood

Since the haemagglutinins of the Rh system are present in human blood only in pathological conditions, it is necessary to depend solely on the reaction of the red corpuscles in order to determine the Rh group of a specimen of blood. Red corpuscles are obtained either by bleeding into a 3.0 per cent w/v solution of *sodium citrate* or by resuspending in a 0.9 per cent w/v solution of *sodium chloride* red corpuscles obtained by breaking up a clot. The suspension in sodium citrate or sodium chloride solution is centrifuged and the corpuscles resuspended in a 0.9 per cent w/v solution of *sodium chloride* to give an approximately 2 per cent v/v suspension of packed corpuscles.

In order to select Rh negative blood, the untested donor blood is first tested with anti-D Rh serum and, in order to conserve these very rare sera, only those bloods which are unagglutinated are further tested with anti-C Rh serum and anti-E Rh serum.

For economical routine work, 0.01 ml volumes of serum and corpuscle suspension are used, though for inexperienced workers, 0.04 ml volumes are preferable. With a Pasteur pipette, graduated at 0.01 ml intervals, 0.01 ml of anti-D Rh serum is deposited at the bottom of 50 mm × 6 mm lipless test-tubes; 0.01 ml of corpuscles suspension is then deposited on the wall of the tube about 4 mm above the serum and the two mixed and incubated for two hours at 37°. Control tests of the serum are made with D-positive and D-negative corpuscles. For reading, the deposited corpuscles are gently taken up with a Pasteur pipette, spread evenly on a slide with the stem of the pipette and examined microscopically at a magnification of ×50 to ×100. D-positive corpuscles are in large clumps, D-negative corpuscles are unagglutinated. The tests with anti-C Rh serum and anti-E Rh serum are made similarly, but, since the agglutination is usually weak, the deposited corpuscles must be transferred to the slide with a minimum of manipulation.

It is advisable to control the specificity of each anti-Rh serum used by testing it with red corpuscles of Rh groups that should react positively and of Rh groups that should react negatively with each serum.

Reagents

(a) ANTI-A SERUM

Source : Anti-A serum is derived from selected B group persons with or without immunisation by group specific red cells or substances or from the sera of lower animals after such immunisation. It may be prepared by reconstituting the dried serum.

Properties : It agglutinates human red cells containing A agglutinogen, i.e. blood groups A and AB including subgroups A_1, A_2, A_1B, A_2B etc., and does not agglutinate human red cells which do not contain A agglutinogen, i.e. blood groups O and B.

Description : Liquid anti-A serum is a clear or slightly opalescent fluid, unless artificially coloured blue or blue green. The dried anti-A serum suitable for preparing the reagent by reconstitution is light yellow to deep cream in colour, unless artificially coloured as indicated for liquid serum. Liquid serum may contain a suitable anti-bacterial preservative.

Potency : The following are the minimum requirements

Serum	Test Corpuscles	Titre
Anti-A	A_1	256
	A_2	128
	A_2B	64

for the titre of anti-A serum with A_1, A_2 and A_2B corpuscles.

Avidity : The following are the minimum requirements for the time taken by anti-A serum to show naked eye agglutination when mixed on a slide with an equal volume of a 5 to 10 per cent suspension of A_1, A_2, and A_2B cells.

Serum	Test Corpuscles	Avidity
Anti-A	A_1	15 seconds
	A_2	30 seconds
	A_2B	45 seconds

Sterility : Complies with the *test for sterility*, Appendix 4.6.

Storage : Anti-A serum should be kept in a sterile container, sealed so as to exclude micro-organisms and protected from light. It should be dispensed with precautions against deterioration and bacterial contamination.

Liquid anti-A serum which does not contain an anti-bacterial preservative should be kept frozen solid, preferably at a temperature below −20°

Liquid anti-A serum containing an anti-bacterial preservative should be kept at a temperature of 4°. Dried anti-A serum should be kept at a temperature not greater than 25°.

Labelling : The label should state: (i) the name Anti-A serum, (ii) the quantity, (iii) the batch number, (iv) the expiry date, (v) the storage conditions, and (vi) the preservative, if any.

(b) ANTI-B SERUM

Source : Anti-B serum is derived from the sera of selected A group persons with or without immunisation by group specific red cells or substances or from the sera of lower animals after such immunisation. It may be prepared by reconstituting the dried serum.

Properties : It agglutinates human red cells containing B agglutinogen, i.e. blood groups B and AB including sub-groups A_1B and A_2B and does not agglutinate human red cells which do not contain B agglutinogens, i.e. blood groups O and A including sub-groups A_1 and A_2.

Description : Liquid anti-B serum is a clear or slightly opalescent fluid unless artificially coloured yellow. The dried anti-B serum suitable for preparing the reagent by reconstitution is light yellow to deep cream in colour unless artificially coloured as indicated for liquid serum. Liquid serum may contain a suitable anti-bacterial preservative.

Potency : The following are the minimum requirements for the titre of an anti-B serum.

Serum	Test Corpuscles	Titre
Anti-B	B	256

Avidity : The following are the minimum requirements for the time taken by anti-B serum to show naked eye agglutination when mixed on a slide with an equal volume of a 5 to 10 per cent suspension of B cells.

Serum	Test Corpuscles	Avidity
Anti-B	B	15 seconds

Sterility : Complies with *the test for sterility*, Appendix 4.6.

Storage : Anti-B serum should be kept in a sterile container, sealed so as to exclude micro-organisms, and protected from light. It should be dispensed with precautions against deterioration and bacterial contamination.

Liquid anti-B serum which does not contain an anti-bacterial preservative should be kept frozen solid preferably at a temperature below −20°

Liquid anti-B serum containing an anti-bacterial preservative should be kept at a temperature of 4°.

Dried anti-B serum should be kept at a temperature not greater than 25°.

Labelling : The label should state: (i) the name Anti-B serum, (ii) the quantity, (iii) batch number, (iv) the expiry date, (v) the storage conditions, and (vi) the preservative, if any.

2.40 PHOTOMETRIC HAEMOGLOBINOMETRY

The determination of haemoglobin in blood is done by the measurement of colour of cyanmethaemoglobin obtained by conversion of haemoglobin to cyanmethaemoglobin by means of a special reagent.

Reagents

Ferricyanide-cyanide reagent : Dissolve 0.2 g of *potassium ferricyanide*, 50 mg of *potassium cyanide* and 0.14 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml. Check the pH and adjust, if necessary, to between 7.0 and 7.4. Store in a polythene bottle in the dark at a temperature below 20°.

NOTE — The solution should not be frozen.

Method

Pipette 0.02 ml of the substance being examined into a stoppered tube, add 4.0 ml of *ferricyanide-cyanide reagent* and mix well. Allow to stand for ten minutes and

measure the *extinction* of a 1-cm layer of the resulting solution at 540 nm, Appendix 5.15 A, using as blank the *ferricyanide-cyanide reagent*. Calculate the content of haemoglobin from the *extinction* obtained by carrying out the determination simultaneously using a suitable volume of *cyanmethaemoglobin R.S.* and from the declared content of haemoglobin in the *cyanmethaemoglobin R.S.*

2.41 TEST FOR HAEMOLYSINS

Add one volume of fresh donor serum to one volume of a 10 per cent w/v suspension of A_1 cells in a 0.9 per cent w/v solution of *sodium chloride* and one volume to one volume of a similar suspension of B cells; a similar test using O cells may be done as a negative control. If the serum is more than twenty-four hours old, add one volume of fresh group O serum free of lysins to each tube as a source of complement. Mix the contents of each tube, incubate at 37° for one hour, and examine the supernatant liquids for haemolysis.

A serum giving a positive result in this test is further examined as follows: Dilute one volume of the serum with three volumes of a 0.9 per cent w/v solution of *sodium chloride* and mix one volume of the diluted serum with one volume of fresh group O serum free of lysins and one volume of a 10 per cent w/v suspension, in a 0.9 per cent w/v solution of *sodium chloride*, of A_1 or B cells (whichever were lysed in the first test). At the same time, in two further tubes, mix one volume of the 0.9 per cent w/v solution of *sodium chloride* with one volume of the fresh group O serum free of lysins. To one of these tubes add one volume of a 10 per cent w/v suspension, in the 0.9 per cent w/v solution of *sodium chloride*, of A_1 cells and to the other one volume of a similar suspension of B cells. Incubate the tubes for one hour at 37°, mix the contents of each tube and examine the supernatant liquids for haemolysis. No haemolysis should show in any of the tubes.

3. Chemical Tests and Assays

3.1 IDENTIFICATION REACTIONS

The following tests may be used for the identification of chemicals referred to in the Pharmacopoeia. They are not intended to be applicable to mixtures of substances unless so specified.

Acetates

(A) Heat the substance being examined with an equal quantity of *oxalic acid*. Acid vapours with the characteristic odour of acetic acid are liberated.

(B) Warm 1 g of the substance being examined with 1 ml of *sulphuric acid* and 3 ml of *alcohol*; ethyl acetate, recognisable by its odour, is evolved.

(C) Dissolve about 30 mg of the substance being examined in 3 ml of *water* or use 3 ml of the prescribed solution, add successively 0.25 ml of *lanthanum nitrate solution*, 0.1 ml of *0.1N iodine* and 0.05 ml of *dilute ammonia solution*. Heat carefully to boiling. Within a few minutes a blue precipitate is formed or a dark blue colour develops.

(D) Heat about 20 mg of the substance being examined with 50 mg of *calcium oxide*; acetone is evolved, which may be detected by the indigo blue colour obtained when vapours impinge on filter paper which has been moistened with a 2 per cent w/v solution of *2-nitrobenzaldehyde* in *alcohol*, dried, and moistened with *N sodium hydroxide*.

Acetyl Groups

In a test-tube, about 180 mm × 18 mm, place 10 to 20 mg of the substance being examined, or the prescribed quantity, and 0.15 ml of *phosphoric acid*. Close the tube with a stopper through which passes a small test-tube, about 100 mm × 10 mm, containing water to act as a condenser. On the outside of the smaller tube, hang a drop of a 5 per cent w/v solution of *lanthanum nitrate*.

Except for substances hydrolysable only with difficulty, place the apparatus in a water-bath for five minutes, and remove the smaller tube. Mix the drop with one drop of *2N ammonia*. After one or two minutes a blue colour appears at the junction of the two drops; the colour intensifies and persists for a short time.

For substances hydrolysable only with difficulty, heat the mixture slowly to boiling point over an open flame instead of using a water-bath.

Alkaloids

Dissolve a few mg of the substance being examined or the prescribed quantity in 5 ml of *water*, add *dilute hydrochloric acid* until the solution has an acid reaction, and then add 1 ml of *potassium iodobismuthate solution*. An orange or orange-red precipitate is formed immediately.

Aluminium

(A) Dissolve about 20 mg of the substance being examined in 2 ml of *water* or use 2 ml of the prescribed solution, add about 0.5 ml of *2N hydrochloric acid* and about 0.5 ml of *thioacetamide reagent*; no precipitate forms. Add dropwise *2N sodium hydroxide solution*; a gelatinous white precipitate appears that redissolves on addition of further *sodium hydroxide solution*. Gradually add *ammonium chloride solution*; the gelatinous white precipitate reappears.

(B) Dissolve about 20 mg of the substance being examined in 5 ml of *water* or use 5 ml of the prescribed solution, add five drops of *ammonium acetate solution* and five drops of a 0.1 per cent w/v solution of *mordant blue 3*; an intense purple colour is produced.

(C) To a solution of the substance being examined, in *water* add *dilute ammonia solution* until a faint precipitate is produced, and then add 0.25 ml of a freshly prepared 0.05 per cent w/v solution of *quinalizarin* in a 1 per cent w/v solution of *sodium hydroxide*. Heat to boiling, cool, and acidify with an excess of *acetic acid*, a reddish-violet colour is produced.

Amines, Primary Aromatic

Acidify the prescribed solution with *2N hydrochloric acid*, or dissolve 0.1 g of the substance being examined in 2 ml of *2N hydrochloric acid*, and add 0.2 ml of *sodium nitrite solution*. After one to two minutes add the solution to 1 ml of *β-naphthol solution*; an intense orange or red colour and usually a precipitate of the same colour is produced.

Ammonium Salts

(A) Heat a few mg of the substance being examined with *sodium hydroxide solution*; ammonia is evolved, which is recognisable by its odour and by its action on moist *red litmus paper*.

(B) To the prescribed solution add 0.2 g of *light magnesium oxide*. Pass a current of air through the mixture and direct the gas that is evolved to just beneath the surface of a mixture of 1 ml of *0.1N hydrochloric acid* and 0.05 ml of *methyl red solution*; the colour of the solution changes to yellow. On addition of 1 ml of a freshly

prepared 10 per cent w/v solution of *sodium cobaltinitrite*, a yellow precipitate forms.

Antimony

Dissolve with gentle heating about 10 mg of the substance being examined in a solution of 0.5 g of *sodium potassium tartrate* in 10 ml of *water* and allow to cool; 2 ml of this solution, or 2 ml of the prescribed solution, to which *sodium sulphide solution* has been added dropwise, gives a reddish-orange precipitate which dissolves on adding *dilute sodium hydroxide solution*.

Arsenic

Heat 5 ml of the prescribed solution on a water-bath with an equal volume of *hypophosphorus reagent*; a brown precipitate is formed.

Barbiturates

Dissolve 5 mg of the substance being examined in 3 ml of a hot 0.2 per cent w/v solution of *cobalt acetate* in *methyl alcohol*, add 5 mg of finely powdered *sodium tetraborate* and boil; a blue-violet colour is produced.

Barbiturates, Non-nitrogen Substituted

Dissolve 5 mg of the substance being examined in 3 ml of *methyl alcohol*, add 0.1 ml of a solution containing 10 per cent w/v of *cobalt nitrate* and 10 per cent w/v of *calcium chloride*, mix and add, with shaking, 0.1 ml of *dilute sodium hydroxide solution*; a violet-blue colour and precipitate are produced.

Barium

(A) Barium salts impart a yellowish-green colour to a nonluminous flame, appearing blue when viewed through green glass.

(B) Dissolve about 20 mg of the substance being examined in 5 ml of *dilute hydrochloric acid*, add 2 ml of *dilute sulphuric acid*; a white precipitate forms which is insoluble in *nitric acid*.

Benzoates

(A) To 1 ml of a 10 per cent w/v neutral solution add 0.5 ml of *ferric chloride test solution*; a dull-yellow precipitate, soluble in *solvent ether*, is formed.

(B) Moisten 0.2 g of the substance being examined with 0.2 ml to 0.3 ml of *sulphuric acid* and gently warm the bottom of the tube; a white sublimate is deposited on the inner walls of the tube and no charring occurs.

(C) Dissolve 0.5 g of the substance being examined in 10 ml of *water*, or use 10 ml of the prescribed solution and add 0.5 ml of *hydrochloric acid*. The precipitate obtained, after crystallisation from *water* and drying under reduced pressure melts at about 122°, Appendix 5.11.

Bicarbonates

(A) Solutions of bicarbonates, when boiled, liberate carbon dioxide.

(B) Treat a solution of the substance being examined with a solution of *magnesium sulphate*; no precipitate is formed (distinction from carbonates). Boil; a white precipitate is formed.

(C) Introduce into a test-tube 0.1 g of the substance being examined suspended in 2 ml of *water* or use the prescribed solution. Add 2 ml of *2N acetic acid*. Close the tube immediately using a stopper fitted with a glass tube bent at two right-angles. Heat gently and collect the gas in 5 ml of *barium hydroxide solution*; a white precipitate forms that dissolves on addition of an excess of *dilute hydrochloric acid*.

Bismuth

(A) To 0.5 g of the substance being examined add 10 ml of *2N hydrochloric acid* or use 10 ml of the prescribed solution. Heat to boiling for one minute, cool, and filter if necessary. To 1 ml of the solution obtained add 20 ml of *water*; a white or slightly yellow precipitate appears which on addition of 0.05 to 0.1 ml of *sodium sulphide solution* turns brown.

(B) To about 50 mg of the substance being examined add 10 ml of *2N nitric acid* or use 10 ml of the prescribed solution. Heat to boiling for one minute, allow to cool and filter if necessary. To 5 ml of the solution obtained add 2 ml of a 10 per cent w/v solution of *thiourea*; an orange-yellow colour or an orange precipitate is produced. Add 4 ml of a 2.5 per cent w/v solution of *sodium fluoride*; the solution is not decolorised within thirty minutes.

Bromides

(A) Dissolve a quantity of the substance being examined, equivalent to about 3 mg of bromide ion in 2 ml of *water* or use 2 ml of the prescribed solution. Acidify with *2N nitric acid* and add 1 ml of *0.1N silver nitrate*. Shake and allow to stand; a curdy, pale-yellow precipitate forms. Centrifuge and wash the precipitate rapidly with three quantities, each of 1 ml, of *water* in subdued light. Suspend the precipitate in 2 ml of *water* and add 1.5 ml of *10N ammonia*; the precipitate dissolves with difficulty.

(B) Dissolve about 10 mg in 2 ml of *water* and add 1 ml of *chlorine solution*; bromine is evolved, which is soluble in two or three drops of *chloroform*, forming a reddish solution. The addition of *phenol solution* to the aqueous solution containing liberated bromine yields a white precipitate.

NOTE—In testing for bromides in the presence of iodides, all iodine must first be removed by boiling the aqueous solution with an excess of lead dioxide.

Calcium

(A) Dissolve 20 mg of the substance being examined in

5 ml of *5M acetic acid* or add 1 ml of *glacial acetic acid* to 5 ml of the prescribed solution. Add 0.5 ml of *potassium ferrocyanide solution*; the solution remains clear. Add about 50 mg of *ammonium chloride*; a white, crystalline precipitate is formed.

(B) To a solution of the substance being examined add a few drops of a solution of *ammonium oxalate*; a white precipitate is obtained that is only sparingly soluble in *dilute acetic acid* but is soluble in *hydrochloric acid*.

(C) Dissolve 20 mg of the substance being examined in the minimum quantity of *dilute hydrochloric acid* and neutralise with *dilute sodium hydroxide solution* or use 5 ml of the prescribed solution; add 5 ml of *ammonium carbonate solution*; a white precipitate is formed which, after boiling and cooling the mixture, is only sparingly soluble in *ammonium chloride solution*.

Carbonates

(A) Suspend 0.1 g of the substance being examined in a test-tube in 2 ml of *water* or use the prescribed solution. Add 2 ml of *2N acetic acid*. Close the tube immediately using a stopper fitted with a glass tube bent at two right-angles. Heat gently and collect the gas in 5 ml of *0.1N barium hydroxide*; a white precipitate is formed that dissolves on addition of an excess of *dilute hydrochloric acid*.

(B) Treat a solution of the substance being examined with a solution of *magnesium sulphate*; a white precipitate is formed (distinction from bicarbonates).

Chlorides

(A) Dissolve in 2 ml of *water* a quantity of the substance being examined equivalent to about 2 mg of chloride ion or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid* and add 0.5 ml of *silver nitrate solution*. Shake and allow to stand; a curdy, white precipitate is formed, which is insoluble in *nitric acid*, but soluble, after being well washed with *water*, in *dilute ammonia solution*, from which it is re-precipitated by the addition of *nitric acid*.

(B) Introduce into a test-tube a quantity of the substance being examined equivalent to about 10 mg of chloride ion, add 0.2 g of *potassium dichromate* and 1 ml of *sulphuric acid*. Place a filter-paper strip moistened with 0.1 ml of *diphenylcarbazide solution* over the opening of the test-tube; the paper turns violet-red. Do not bring the moistened paper into contact with the potassium dichromate solution.

Citrates

(A) To a neutral solution of the substance being examined add a solution of *calcium chloride*; no precipitate is produced. Boil the solution; a white precipitate, soluble in *acetic acid*, forms.

(B) Dissolve in 5 ml of *water* a quantity of the substance being examined equivalent to about 50 mg of citric acid or use 5 ml of the prescribed solution. Add 0.5 ml of *sulphuric acid* and 3 ml of *potassium permanganate solution*. Warm until the colour of the permanganate is discharged and add 0.5 ml of a 10 per cent w/v solution of *sodium nitroprusside* in *2N sulphuric acid* and 4 g of *sulphamic acid*. Make alkaline with *strong ammonia solution*, added dropwise, until all the sulphamic acid has dissolved; further addition of *strong ammonia solution* produces a violet colour, turning to violet-blue.

Ferric Salts

(A) Dissolve a quantity of the substance being examined equivalent to about 10 mg of iron in 1 ml of *water* or use 1 ml of the prescribed solution. Add 1 ml of a 5 per cent w/v solution of *potassium ferrocyanide*; an intense blue precipitate is formed that is insoluble in *dilute hydrochloric acid*.

(B) To 3 ml of a solution containing about 0.1 mg of iron or to 3 ml of the prescribed solution, add 1 ml of *2N hydrochloric acid* and 1 ml of *ammonium thiocyanate solution*; the solution becomes blood-red in colour. Take two portions, each of 1 ml, of the mixture. To one portion add 5 ml of *solvent ether*, shake and allow to stand; the ether layer is pink. To the other portion add 3 ml of *0.2M mercuric chloride*; the red colour disappears.

(C) To 2 ml of a solution containing about 0.1 mg of iron or to 3 ml of the prescribed solution add *acetic acid* until the solution is strongly acidic and add 2 ml of a 0.2 per cent w/v solution of *8-hydroxy-7-iodoquinoline-5-sulphonic acid*. A stable green colour is produced.

Ferrous Salts

(A) Dissolve a quantity of the substance being examined corresponding to about 10 mg of iron in 2 ml of *water* or use 2 ml of the prescribed solution. Add 2 ml of *dilute sulphuric acid* and 1 ml of a 0.1 per cent w/v solution of *1,10-phenanthroline*. An intense red colour is produced; the colour is discharged by addition of a slight excess of *0.1N ceric ammonium sulphate*.

(B) To 1 ml of a solution containing not less than 1 mg of iron or to 1 ml of the prescribed solution, add 1 ml of *potassium ferricyanide solution*; a dark blue precipitate is formed that is insoluble in *dilute hydrochloric acid* and is decomposed by *sodium hydroxide solution*.

(C) To 1 ml of a solution containing not less than 1 mg of iron or to 1 ml of the prescribed solution, add 1 ml of *potassium ferrocyanide solution*; a white precipitate is formed which rapidly becomes blue and is insoluble in *dilute hydrochloric acid*.

Iodides

(A) Dissolve a quantity of the substance being examined equivalent to about 4 mg of iodide ion in 2 ml of

water or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid* and add 0.5 ml of *silver nitrate solution*. Shake and allow to stand, a curdy, pale-yellow precipitate forms. Centrifuge and wash the precipitate rapidly with three quantities, each of 1 ml of *water* in subdued light. Suspend the precipitate in 2 ml of *water* and add 1.5 ml of *10N ammonia*; the precipitate does not dissolve.

(B) To 0.2 ml of a solution of the substance being examined containing the equivalent of about 5 mg of iodide ion per ml or to 0.2 ml of the prescribed solution, add 0.5 ml of *2N sulphuric acid*, 0.15 ml of *potassium dichromate solution*, 2 ml of *water* and 2 ml of *chloroform*. Shake for a few seconds and allow to stand; the chloroform layer is violet or violet-red.

(C) To 1 ml of a solution of the substance being examined containing the equivalent of about 5 mg of iodide ion, add 0.5 ml of *mercuric chloride solution*; a dark red precipitate is formed which is slightly soluble in an excess of this reagent and very soluble in an excess of *potassium iodide solution*.

Lactates

To 5 ml of a solution of the substance being examined containing the equivalent of about 5 mg of lactic acid, or to 5 ml of the prescribed solution, add 1 ml of *bromine water* and 0.5 ml of *2N sulphuric acid*. Heat on a water-bath until the colour is discharged, stirring occasionally with a glass rod. Add 4 g of *ammonium sulphate*, mix and add dropwise, without mixing, 0.2 ml of a 10 per cent w/v solution of *sodium nitroprusside* in *2N sulphuric acid*. Still without mixing add 1 ml of *strong ammonia solution* and allow to stand for thirty minutes; a dark green ring appears at the interface of the two liquids.

Lead

(A) Dissolve 0.1 g of the substance being examined in 1 ml of *dilute acetic acid* or use 1 ml of the prescribed solution. Add 2 ml of *potassium chromate solution*; a yellow precipitate forms that is insoluble in 2 ml of *sodium hydroxide solution*.

(B) Dissolve 50 mg of the substance being examined in 1 ml of *dilute acetic acid* or use a 0.5 ml of the prescribed solution. Add 10 ml of *water* and 0.2 ml of *M potassium iodide*; a yellow precipitate forms. Heat to boiling for one or two minutes, and allow to cool; the precipitate is reformed as glistening, yellow plates.

Magnesium

(A) Dissolve about 15 mg of the substance being examined in 2 ml of *water* or use 2 ml of the prescribed solution. Add 1 ml of *dilute ammonia solution*; a white precipitate forms that is redissolved by adding 1 ml of *2M ammonium chloride*. Add 1 ml of *0.25M disodium hydrogen phosphate*; a white crystalline precipitate forms.

(B) To 0.5 ml of a neutral or slightly acidic solution of the substance being examined add 0.2 ml of a 0.1 per cent w/v solution of *titan yellow* and 0.5 ml of *0.1N sodium hydroxide*; a bright red turbidity develops which gradually settles to give a bright red precipitate.

Nitrates

(A) Dissolve 15 mg of the substance being examined in 0.5 ml of *water*, add cautiously 1 ml of *sulphuric acid*, mix, and cool. Incline the tube and carefully add, without mixing, 0.5 ml of *ferrous sulphate solution*; a brown colour forms at the interface of the two liquids.

(B) To a mixture of 0.1 ml of *nitrobenzene* and 0.2 ml of *sulphuric acid* add a quantity of the powdered substance equivalent to about 1 mg of nitrate ion or the prescribed quantity. Allow to stand for five minutes and cool in ice water whilst adding slowly with stirring 5 ml of *water* and then 5 ml of *sodium hydroxide solution*. Add 5 ml of *acetone*, shake, and allow to stand, the upper layer shows an intense violet colour.

Penicillins

To 2 mg of the substance being examined add 2 mg of *chromotropic acid sodium salt* and 2 ml of *sulphuric acid* and immerse in an oil-bath at 150°; the solution, when shaken and examined every thirty seconds, exhibits the colours stated in Table 1.

Phosphates (Orthophosphates)

(A) To 5 ml of the prescribed solution, neutralised to pH 7.0, add 5 ml of *silver nitrate solution*; a light yellow precipitate forms the colour of which is not changed by boiling and which is readily soluble in *dilute ammonia solution* and in *dilute nitric acid*.

(B) Mix 1 ml of the prescribed solution with 1 ml of *ammoniacal magnesium sulphate solution*; a white, crystalline precipitate is produced.

(C) To 2 ml of the prescribed solution, add 2 ml of *dilute nitric acid* and 4 ml of *ammonium molybdate solution* and warm the solution; a bright canary-yellow precipitate is formed.

Potassium

(A) Dissolve about 50 mg of the substance being examined in 1 ml of *water* or use 1 ml of the prescribed solution. Add 1 ml of *dilute acetic acid* and 1 ml of a freshly prepared 10 per cent w/v solution of *sodium cobaltinitrite*; a yellow or orange-yellow precipitate forms immediately.

(B) Dissolve 0.1 g of the substance being examined in 2 ml of *water* or use 2 ml of the prescribed solution. Heat the solution with 1 ml of *sodium carbonate solution*; no precipitate forms. Add 0.05 ml of *sodium sulphide solution*, no precipitate forms. Cool in ice water and add 2

TABLE 1

Time (min)	Ampicillin, Ampicillin Sodium, Ampicillin Trihydrate	Benzathine Penicillin, Benzyl-penicillin Potassium	Carbenicillin Sodium	Cloxacillin Sodium	Phenoxymethyl-penicillin Potassium
0	Colourless	Yellow	Colourless	Colourless	Colourless
0.5	Colourless	Yellow	Light-brown	Pale-yellow	Colourless
1	Colourless	Yellow	Yellowish-brown	Greenish-yellow	Colourless
1.5	Colourless	Orange-yellow	Greenish-brown	Yellowish-green	Pale pink
2	Purple	Orange-yellow	Greenish-brown	Green	Purple
2.5	Deep purple	Orange-yellow	Brown	Greenish-purple	Purple
3	Violet	Pale orange	Dark brown	Purple	Bluish-violet
3.5	Violet	Orange or may char	Dark brown	Purple	Dark blue
4	Charred		Dark brown	Purple	Dark blue

ml of a 15 per cent w/v solution of *tartaric acid* and allow to stand; a white, crystalline precipitate forms.

(C) Ignite a few mg of the substance being examined, cool and dissolve in the minimum quantity of *water*. To this solution add 1 ml of *platinic chloride solution* in the presence of 1 ml of *hydrochloric acid*; a yellow, crystalline precipitate forms, which on ignition leaves a residue of potassium chloride and platinum.

Salicylates

(A) To 1 ml of a 10 per cent w/v neutral solution add 0.5 ml of *ferric chloride test solution*; a violet colour is produced which persists after the addition of 0.1 ml of *dilute acetic acid*.

(B) Dissolve 0.5 g of the substance being examined in 10 ml of *water* or use 10 ml of the prescribed solution. Add 0.5 ml of *hydrochloric acid*; the precipitate obtained after recrystallisation from hot *water* and drying "in vacuo", melts at about 159°, Appendix 5.11.

(C) Dissolve 0.5 g of the substance being examined in 10 ml of *water* or use 10 ml of the prescribed solution and add 2 ml of *bromine solution*; a cream-coloured precipitate is formed.

Silver

(A) Dissolve 10 mg of the substance being examined in 10 ml of *water* or use 10 ml of the prescribed solution Add 0.3 ml of *dilute hydrochloric acid*; a curdy, white precipitate is formed that is soluble in *dilute ammonia solution*. Add *potassium iodide solution*; a yellow precipitate is formed.

(B) Dissolve 10 mg of the substance being examined in 10 ml of *water* or use 10 ml of the prescribed solution and add 2 ml of *potassium chromate solution*; a red precipitate is formed which is soluble in *nitric acid*.

Sodium

(A) Dissolve 0.1 g of the substance being examined in 2 ml of *water* or use 2 ml of the prescribed solution. Add 2 ml of a 15 per cent w/v solution of *potassium carbonate* and heat to boiling; no precipitate forms. Add 4 ml of freshly prepared *potassium antimonate solution* and heat to boiling. Allow to cool ice water and if necessary rub the inside of the test-tube with a glass rod; a dense, white precipitate is formed.

(B) Acidify a solution of the substance being examined with *N acetic acid* and add a large excess of *magnesium uranyl acetate solution*; a yellow, crystalline precipitate is formed.

Sulphates

(A) Dissolve about 50 mg of the substance being examined in 5 ml of *water* or use 5 ml of the prescribed solu-

tion. Add 1 ml of *dilute hydrochloric acid* and 1 ml of *barium chloride solution*; a white precipitate forms.

(B) Dissolve about 50 mg of the substance being examined in 5 ml of *water* or use 5 ml of the prescribed solution and add 2 ml of *lead acetate solution*; a white precipitate is formed which is soluble in *ammonium acetate solution* and in *sodium hydroxide solution*.

(C) Add 0.1 ml of *iodine solution* to the suspension obtained in Test A; the suspension remains yellow (distinction from sulphites and dithionites) but is decolorised by adding, dropwise *stannous chloride solution* (distinction from iodates) Boil the mixture; no coloured precipitate appears (distinction from selenates and fungstates).

Tartrates

(A) Warm the substance being examined with *sulphuric acid*; charring occurs and carbon monoxide, which burns with a blue flame when ignited, is evolved.

(B) Dissolve about 20 mg of the substance being examined in 5 ml of *water* or use 5 ml of the prescribed solution. Add 0.05 ml of a 1 per cent w/v solution of *ferrous sulphate* and 0.05 ml of *hydrogen peroxide solution*; a transient yellow colour is produced. Add *2N sodium hydroxide solution* dropwise; an intense blue colour is produced.

(C) Heat 0.1 ml of a solution containing the equivalent of about 2 mg of tartaric acid or 0.1 ml of the prescribed solution on a water-bath for five to ten minutes with 0.1 ml of a 10 per cent w/v solution of *potassium bromide*, 0.1 ml of a 2 per cent w/v solution of *resorcinol* and 3 ml of *sulphuric acid*; a dark-blue colour is produced that changes to red when the solution is cooled and poured into *water*.

Thiosulphates

(A) Dissolve 0.1 g of the substance being examined in 5 ml of *water*, add 2 ml of *hydrochloric acid*; a white precipitate is formed which soon turns yellow and sulphur dioxide is evolved, recognisable by its odour.

(B) Dissolve 0.1 g of the substance being examined in 5 ml of *water*, add 2 ml of *ferric chloride-test solution*; a dark violet colour is produced which quickly disappears.

(C) Solutions of thiosulphates decolorise *iodine solution*; the decolorised solutions do not give the reactions of *sulphates*.

(D) Solutions of thiosulphates decolorise *bromine solution*; the decolorised solutions give the reactions of *sulphates*.

Zinc

(A) Dissolve 0.1 g of the substance being examined in 5 ml of *water*; or use 5 ml of the prescribed solution, Add 0.2 ml of *sodium hydroxide solution*; a white precipitate forms. Add a further 2 ml of *sodium hyaroxide solution*; the precipitate dissolves. Add 10 ml of *ammonium chloride solution*; the solution remains clear, but a flocculent, white precipitate forms on addition of 0.1 ml of *sodium sulphide solution*.

(B) Dissolve 0.1 g of the substance being examined in 5 ml of *water*, or use 5 ml of the prescribed solution, acidify with *dilute sulphuric acid* and add one drop of a 0.1 per cent w/v solution of *copper sulphate* and 2 ml of *ammonium mercuri-thiocyanate solution*; a violet precipitate is formed.

(C) Dissolve 0.1 g of the substance being examined in 5 ml of *water*, or use 5 ml of the prescribed solution, add 2 ml of *potassium ferrocyanide solution*; a white precipitate is formed which is insoluble in *dilute hydrochloric acid*.

3.2 LIMIT TESTS

3.2.1 Limit Test for Arsenic

In the limit test for arsenic, the amount of arsenic present is expressed as arsenic, As.

Apparatus

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid, and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded or ground smooth.

Two rubber bungs (about 25 mm × 25 mm), each with a hole bored centrally and true, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively the two bungs may be replaced by any suitable contrivance satisfying the conditions described under the **General test.**

Reagents

Ammonium oxalate AsT : *Ammonium oxalate* which complies with the following additional test:

Heat 5 g with 15 ml of *water*, 5 ml of *nitric acid AsT*, and 10 ml of *sulphuric acid AsT* in a narrow-necked, round-bottomed flask until frothing ceases, cool, and apply the **General test**; no visible stain is produced.

Arsenic solution, dilute, AsT :

Strong arsenic solution AsT	1 ml
Water	sufficient to produce 100 ml

Dilute arsenic solution AsT must be freshly prepared. 1 ml contains 0.01 mg of arsenic, As.

Arsenic solution, strong, AsT :

Arsenic trioxide	0.132 g
Hydrochloric acid	50 ml
Water	sufficient to produce 100 ml

Brominated hydrochloric acid AsT :

Bromine solution AsT	1 ml
Hydrochloric acid AsT	100 ml

Bromine solution AsT :

Bromine	30 g
Potassium bromide	30 g
Water	sufficient to produce 100 ml

It complies with the following test :

Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of *water*, 10 ml of *hydrochloric acid AsT* and sufficient *stannous chloride solution AsT* to reduce the remaining bromine and apply the **General test**; the stain produced is not deeper than a 1-ml *standard stain*, showing that the proportion of arsenic present does not exceed 1 part per million.

Citric acid AsT : *Citric acid* which complies with the following additional test: Dissolve 10 g in 50 ml of *water*, add 10 ml of *stannated hydrochloric acid AsT* and apply the **General test**; no visible stain is produced.

Hydrochloric acid AsT : *Hydrochloric acid* diluted with *water* to contain about 32 per cent w/w of HCl and complying with the following additional tests:

(i) Dilute 10 ml with sufficient *water* to produce 50 ml, add 5 ml of *ammonium thiocyanate solution*, and stir immediately; no colour is produced.

(ii) To 50 ml add 0.2 ml of *bromine solution AsT*, evaporate on a water-bath until reduced to 16 ml, adding more *bromine solution AsT*, if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation; add 50 ml of *water* and 5 drops of *stannous chloride solution AsT*, and apply the **General test**; the stain produced is not deeper than a 0.2 ml *standard stain* prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 part per million.

Hydrochloric acid (constant-boiling composition) AsT : Boil *hydrochloric acid AsT* to constant boiling composition in the presence of *hydrazine hydrate*, using 1 ml of a 10 per cent w/v solution in *water* per litre of the acid.

Mercuric chloride paper : Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of *mercuric chloride*, pressed to remove superfluous solution, and dried at about 60° in the dark. The grade of the filter paper is such that the weight is between 65 and 120 g per sq. mm; the thickness in mm of 400 papers is approximately equal, numerically, to the weight in g per sq. m.

NOTE – Mercuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia affords a lighter stain or no stain at all when employed in the limit test for arsenic.

Nitric acid AsT : *Nitric acid* which complies with the following additional test:

Heat 20 ml in a porcelain dish with 2 ml of *sulphuric acid AsT* until white fumes are given off. Cool, add 2 ml of *water*, and again heat until white fumes are given off; cool, add 50 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*, and apply the **General test**; no visible stain is produced.

Potassium chlorate AsT : *Potassium chlorate* which complies with the following additional test:

Mix 5 g in the cold with 20 ml of *water* and 22 ml of *hydrochloric acid AsT*; when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of *stannous chloride solution AsT*, add 20 ml of *water*, and apply the **General test**; no visible stain is produced.

Potassium iodide AsT : *Potassium iodide* which complies with the following additional test:

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water*, add 2 drops of *stannous chloride solution AsT* and apply the **General test**; no visible stain is produced.

Sodium carbonate, anhydrous, AsT : *Anhydrous sodium carbonate* which complies with the following additional test:

Dissolve 5 g in 50 ml of *water*, add 20 ml of *brominated hydrochloric acid AsT*, remove the excess of bromine with a few drops of *stannous chloride solution AsT*, and apply the **General test**; no visible stain is produced.

Stannated hydrochloric acid AsT :

Stannous chloride solution AsT	1 ml
Hydrochloric acid AsT	100 ml

Stannous chloride solution AsT : Prepared from *stannous chloride solution* by adding an equal volume of *hydrochloric acid*, boiling down to the original volume, and filtering through a fine-grain filter paper.

It complies with the following test :

To 10 ml add 6 ml of *water* and 10 ml of *hydrochloric acid AsT*, distil, and collect 16 ml. To the distillate add 50 ml of *water* and 2 drops of *stannous chloride solution AsT* and apply the **General test**; the stain produced is not deeper than a 1-ml *standard stain*, showing that the proportion of arsenic present does not exceed 1 part per million.

Sulphuric acid AsT : *Sulphuric acid* which complies with the following additional test :

Dilute 10 g with 50 ml of *water*, add 0.2 ml of *stannous chloride solution AsT*, and apply the **General test**; no visible stain is produced.

Zinc AsT : *Granulated zinc* which complies with the following additional tests :

Add 10 ml of *stannated hydrochloric acid AsT* to 50 ml of *water*, and apply the **General test**, using 10 g of the zinc and allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of *dilute arsenic solution AsT*; a faint but distinct yellow stain is produced (test for sensitivity).

General Method of Testing

By a variable method of procedure, suitable to the particular needs of each substance, a solution is prepared from the substance being examined which may or may not contain that substance; but contains the whole of the arsenic (if any) originally present in that substance. This solution, referred to as the 'test solution', is used in the actual test.

General test : The glass tube is lightly packed with cotton wool, previously moistened with *lead acetate solution* and dried, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a rounded-off end, or so that the ground end of the tube is flush with the larger end of the bung. A piece of *mercuric chloride paper* is placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band or spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube 6.5 m in diameter interrupted by a diaphragm of *mercuric chloride paper*.

Instead of this method of attaching the *mercuric chloride paper*, any other method may be used provided (1) that the whole of the evolved gas passes through the paper; (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and (3) that the paper is protected from sunlight during the test. The test solution prepared as specified, is placed in the wide-mouthed bottle, 1 g of *potassium iodide AsT* and 10 g of *zinc AsT* are added, and the prepared glass tube is placed quickly in position. The action is allowed to proceed for forty minutes. The yellow stain which is produced on the *mercuric chloride paper* if arsenic is present is compared by daylight with the *standard stains* produced by operating in a similar manner with known quantities of *dilute arsenic solution AsT*. The comparison of the stains is made immediately at the completion of the test. The *standard stains* used for comparison are freshly prepared; they fade on keeping.

By matching the depth of colour with *standard stains*, the proportion of arsenic in the substance may be determined. A stain equivalent to the 1-ml *standard stain* produced by operating on 10 g a substance indicates that the proportion of arsenic is 1 part per million.

NOTES – (1) The action may be accelerated by placing the apparatus on a warm surface, care being taken that the mercuric chloride paper remains dry throughout the test.

(2) The most suitable temperature for carrying out the test is generally about 40°, but because the rate of evolution of the gas varies somewhat with different batches of zinc AsT, the temperature may be adjusted to obtain a regular, but not violent, evolution of gas.

(3) The tube must be washed with hydrochloric acid AsT, rinsed with water, and dried between successive tests.

Standard stains : Solutions are prepared by adding to 50 ml of *water*, 10 ml of *stannated hydrochloric acid AsT* and quantities of *dilute arsenic solution AsT* varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the **General test**, yield stains on the *mercuric chloride paper* referred to as the *standard stains*.

Preparation of the Test Solution

In the various methods of preparing the *test solution* given below, the quantities are so arranged, unless otherwise stated, that when the stain produced from the solution to be examined is not deeper than the 1-ml *standard stain*, the proportion of arsenic present does not exceed the permitted limit.

Aluminium hydroxide gel : Dissolve 10 g in 18 ml of *brominated hydrochloric acid AsT* and 42 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Dried aluminium hydroxide gel : Treat 2 g as described under Aluminium Hydroxide Gel.

Aluminium sulphate : Dissolve 3.3 g in 50 ml of *water*, and add 10 ml of *stannated hydrochloric acid AsT*.

Ammonium chloride : Dissolve 2.5 g in 50 ml of *water*, and add 10 ml of *stannated hydrochloric acid AsT*.

Analgin : Treat 10 g as described under Ammonium Chloride.

Aspirin : Mix 5 g with 3 g of *anhydrous sodium carbonate AsT*, add 10 ml of *bromine solution AsT* and mix

thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid AsT* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*.

Barium sulphate : Disperse 10 g in 50 ml of *water*, and add 10 ml of *stannated hydrochloric acid AsT*.

Benzoic acid : Treat 5 g as described under Aspirin.

Boric acid : Dissolve 10 g with 2 g of *citric acid AsT* in 50 ml of *water*, and add 12 ml of *stannated hydrochloric acid AsT*.

Caffeine : Treat 3.3 g as described under Aspirin.

Calamine : Dissolve 1.25 g in 15 ml of *brominated hydrochloric acid AsT* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Calcium aminosalicylate : Mix 5 g with 10 ml of *bromine solution AsT* and evaporate to dryness on a water-bath. Ignite gently, dissolve the cooled residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT*, and remove the excess of bromine with 2 ml of *stannous chloride solution AsT*.

Calcium Carbonate : Treat 2.5 g as described under Calamine.

Calcium chloride : Treat 3.33 g as described under Aluminium Sulphate.

Calcium gluconate : Dissolve 5 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid AsT*.

Calcium hydroxide : Treat 2.5 g as described under Aluminium Hydroxide Gel.

Calcium lactate : Treat 5 g as described under Calcium Gluconate.

Calcium levulinate : Treat 3.3 g as described under Calcium Gluconate.

Dibasic calcium phosphate : Treat 1 g as described under Calamine

Tribasic calcium phosphate : Treat 2 g as described under Calamine.

Microcrystalline cellulose : Treat 5 g as described under Aspirin.

Citric acid : Treat 10 g as described under Ammonium Chloride.

Anhydrous citric acid : Treat 10 g as described under Ammonium Chloride.

Crystal-violet : Mix 2 g with 200 ml of *water* in a long-necked, round-bottomed flask, add 15 ml of *nitric acid AsT*, bring gently to the boil and continue the boiling until the volume of the liquid is reduced to about 20 ml. Allow to cool, add 10 ml, or a sufficient quantity, of *sulphuric acid AsT* and mix. Heat to boiling and add small successive quantities of *nitric acid AsT*, cooling before each addition, until a colourless liquid is obtained. Heat until white fumes are evolved and if darkening occurs at this stage continue the treatment with *nitric acid AsT*, finally heating until white fumes are again evolved. Allow the colourless liquid to cool, and 25 ml of a saturated solution of *ammonium oxalate AsT*, and boil until the slight froth completely subsides. Cool and dilute to 40 ml with *water*.

To 20 ml of tnis solution add 10 ml of *stannated hydrochloric acid AsT*, distil 20 ml, and add to the distillate 3 drops of *stannous chloride solution AsT* and 40 ml of *water*.

Dextrose : Treat 10 g as described under Ammonium Chloride.

Ferrous fumarate : Mix 2 g with 1.5 g of *anhydrous sodium carbonate AsT*, add 10 ml of *bromine solution AsT* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolved the cooled residue in 20 ml of *brominated hydrochloric acid AsT* and 10 ml of *water*. Transfer to a small flask, add sufficient *stannous chloride solution AsT* to remove the yellow colour, connect to a condenser, and distil 22 ml; to the distillate add 40 ml of *water* and three drops of *stannous chloride solution AsT*.

Ferrous gluconate : To 5 g add 15 ml of *water* and 15 ml of *stannated hydrochloric acid AsT*, distil 22 ml, and add to the distillate 40 ml of *water* and 0.2 ml of *stannous chloride solution AsT*.

Ferrous sulphate : Dissolve 5 g in 10 ml of *water* and 15 ml of *stannated hydrochloric acid AsT* and distil 20 ml; to the distillate add a few drops of *bromine solution AsT*; remove the excess of bromine with a few drops of *stannous chloride AsT* and add 40 ml of *water:*

Dried ferrous sulphate : Treat 3.3 g as described under Ferrous Sulphate.

Gelatin : To 5 g add 10 ml of *water* and allow to stand for one hour. Warm to dissolve and add 10 ml of *hydrochloric acid AsT* and a slight excess of *bromine solution AsT*. Add 2 ml of *stannated hydrochloric acid AsT*, heat under a reflux condenser for one hour, cool, and add 10 ml of *water* and 10 ml of *hydrochloric acid AsT*.

Glycerin : Treat 5 g as described under Aluminium Sulphate.

Guar gum : Treat 3.3 g as described under Aspirin.

Hydrochloric acid : Mix 10 g with 40 ml of *water* and 1 ml of *stannous chloride solution AsT*.

Iron and ammonium citrate : Mix 2.5 g with 1.5 g of *anhydrous sodium carbonate AsT*, add 10 ml of *bromine solution AsT* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 20 ml of *brominated hydrochloric acid AsT* and 10 ml of *water*. Transfer to a small flask, add sufficient *stannous chloride solution AsT* to remove the yellow colour, connect to a condenser, and distil 22 ml; to the distillate add 40 ml of *water* and 3 drops of *stannous chloride solution AsT*

Iron dextran injection : To 5 g in a round-bottomed, long-necked flask add 10 ml of *water* and 10 ml of **nitric acid AsT* and heat until the vigorous evolution of brown fumes ceases. Cool, add 10 ml of **sulphuric acid AsT* and heat again until fumes are evolved, adding *nitric acid AsT* dropwise from time to time until oxidation is complete. Cool, add 30 ml of *water*, bring to boil, and continue boiling until the volume of liquid is reduced to about 20 ml; cool and dilute to 50 ml with *water*. Use aliquot portions of this solution for the *limit test for arsenic* and for the *limit test for heavy metals*, Appendix 3.2.4.

*For this purpose the reagent must also comply with the additional test given in *limit test for lead*, Special Reagent, Appendix 3.2.6.

Gently boil 40 ml of this solution until the volume is reduced to about 15 ml, cool and add 15 ml of *stannated hydrochloric acid AsT* and 3 ml of *stannous chloride solution AsT*. Connect to a condenser and distil 15 ml into 25 ml of *water*. To the distillate add a few drops of *bromine solution AsT*, remove the excess of bromine with a few drops of *stannous chloride solution AsT*, and add 20 ml of *water*. Use the 0.8 ml standard stain for comparison.

Isosorbide dinitrate, diluted : Disperse 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*.

Heavy kaolin : Treat 5 g as described under Barium Sulphate.

Light kaolin : Treat 5 g as described under Barium Sulphate.

Lactic acid : Mix 10 g with 50 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*.

Lactose : Treat 10 g as described under Aluminium Sulphate.

Lithium carbonate : Dissolve 5 g in 15 ml of *brominated hydrochloric acid AsT* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Milk of magnesia : Mix 10 g with 40 ml of *water* and 11 ml of *brominated hydrochloric acid AsT* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Magnesium chloride : Treat 5 g as described under Ammonium Chloride.

Heavy magnesium carbonate : Treat 5 g as described under Lithium Carbonate.

Light magnesium carbonate : Treat 5 g as described under Lithium Carbonate.

Heavy magnesium oxide : Treat 2 g as described in Aluminium Hydroxide Gel.

Light magnesium oxide : Treat 2 g as described in Aluminium Hydroxide Gel.

Magnesium sulphate : Treat 5 g as described under Aluminium Sulphate.

Magnesium trisilicate : Treat 2.5 g as described under Barium Sulphate.

Malt extract : Dissolve 10 g in 10 ml of *water*, add 10 ml of *brominated hydrochloric acid AsT*, and allow to stand for five minutes; remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Mannitol : Treat 5 g as described under Aluminium Sulphate.

Mephenesin : Mix 1 g with 50 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*.

Oxyphenonium bromide : Treat 5 g as described under Aspirin.

Pectin : Mix 3.3 g with 10 ml of *water* and 5 ml of *brominated hydrochloric acid AsT* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Phosphoric acid : Treat 5 g as described under Aluminium Sulphate.

Phthalylsulphathiazole : Treat 5 g as described under Succinylsulphathiazole.

Polysorbate 20 : Heat gently 5 g with 2 ml of *nitric acid AsT* and 0.5 ml of *sulphuric acid AsT*, in a long-necked flask, until the first reaction has subsided, cool, add carefully and in small portions, 15 ml of *nitric acid AsT* and 6 ml of *sulphuric acid AsT*, taking care to avoid excessive foaming, and continue heating, adding further small portions of *nitric acid AsT*, if necessary, until white fumes are evolved and the solution becomes colourless or almost colourless. Cool, add carefully 10 ml of *water*, evaporate until white fumes are evolved, and repeat the addition of *water* and evaporation until all the nitric acid has been removed, cool, dilute to 50 ml with *water*, and add 10 ml of *stannated hydrochloric acid AsT*.

Use the 0.5 ml standard stain for comparison.

Polysorbate 80 : Treat 5 g as described under Polysorbate 20.

Polyethyleneglycol 1500

Polyethyleneglycol 4000

Polyethyleneglycol 6000 : Treat 3.3 g as described under Aspirin.

Polyvinylpyrrolidone : Treat 5 g as described under Aspirin.

Potassium bromide : Treat 5 g as described under Calcium Gluconate.

Potassium chloride : Treat 10 g as described under Aluminium Sulphate.

Potassium citrate : Dissolve 5 g in 50 ml of *water*, and add 15 ml of *stannated hydrochloric acid AsT*.

Potassium iodide : Treat 5 g as described under Calcium Gluconate.

Propylene glycol : Treat 2 g as described under Polysorbate 20.

Propylthiouracil : Treat 2 g as described under Aluminium Sulphate.

Saccharin : Mix 5 g with 3 g of *anhydrous sodium carbonate AsT*, add 10 ml of *bromine solution AsT* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and add to the cooled residue a mixture of 16 ml of *brominated hydrochloric acid AsT* and 5 ml of *bromine solution AsT*. Add 40 ml of *water* and boil gently, adding sufficient *bromine solution AsT* from time to time to maintain a slight excess. Filter and remove the excess of bromine with a sufficient quantity of *stannous chloride solution AsT*.

Saccharin sodium : Treat 5 g as described under Saccharin.

Shellac : Treat 5 g as described under Polysorbate 20.

Sodium acetate : Treat 5 g as described under Potassium Citrate.

Sodium acid phosphate : Treat 5 g as described under Aluminium sulphate.

Sodium antimony gluconate : Dissolve 5 g in 10 ml of *water* and 160 ml of *stannated hydrochloric acid AsT* in a flask, connect to a condenser and distil 20 ml; wash the flask and condenser, return the distillate to the flask, add one drop of *stannous chloride solution AsT*, and redistil 16 ml; to the distillate add 45 ml of *water* and two drops of *stannous chloride solution AsT*.

Sodium benzoate : Treat 5 g as described under Sodium Salicylate.

Sodium bicarbonate : Dissolve 5 g in 50 ml of *water*, add 15 ml of *brominated hydrochloric acid AsT*, and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Sodium carboxymethylcellulose : Place 5 g in a dry Kjeldahl flask, add 20 ml of *nitric acid AsT*, and warm cautiously until the reaction commences. Allow the reaction to subside without further heating, then add a mixture of 20 ml of *nitric acid AsT* and 5 ml of *sulphuric acid AsT* and heat until brown fumes cease to be evolved. Add 0.5 ml of *perchloric acid (60 per cent)*, heat until white fumes appear, and if the liquid is still dark add further small quantities of *nitric acid AsT* and heat until the liquid becomes pale yellow. Heat again until white fumes appear and continue heating for a further fifteen minutes. Add 0.5 ml of *perchloric acid (60 per cent)* and continue heating for a few minutes. Allow the solution to cool, add 10 ml of *water*, and heat until white fumes appear. Repeat the heating with a further 5 ml of *water*, cool, and add 40 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*. Use the 0.5 ml *standard stain* for comparison.

Sodium chloride : Treat 10 g as described under Calcium Gluconate.

Sodium chloride hypertonic injection : To 50 ml add 12 ml of *stannated hydrochloric acid AsT*.

Compound sodium chloride injection : Evaporate 100 ml to a volume of about 50 ml and add 12 ml of *stannated hydrochloric acid AsT*.

Compound sodium chloride solution : Treat 100 ml as described under Compound Sodium Chloride Injection.

Sodium citrate : Dissolve 5 g in 50 ml of *water* and add 15 ml of *stannated hydrochloric acid AsT*.

Sodium hydroxide : Dissolve 2.5 g in 50 ml of *water*, add 16 ml of *brominated hydrochloric acid AsT*, and remove the excess of bromine with a few drops of *stannous chloride solution AsT*.

Sodium metabisulphite : Mix 2.5 g in a porcelain dish with 10 ml of *water*, 1.25 g of *potassium chlorate AsT*, and 16 ml of *hydrochloric acid AsT*, and heat to expel chlorine, remove the last traces of chlorine with a few drops of *stannous chloride solution AsT* and add 35 ml of *water*.

Sodium phosphate : Treat 2 g as described under Aluminium Sulphate.

Sodium salicylate : Mix 5 g with 10 ml of *bromine solution AsT* and evaporate to dryness on a water-bath. Ignite gently, dissolve the cooled residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT*, and remove the excess of bromine with 2 ml of *stannous chloride solution AsT*.

Sodium thiosulphate : Treat 5 g as described under Aluminium Sulphate.

Sorbitol : Treat 5 g as described under Aluminium Sulphate.

Sorbitol solution : Dissolve 3.3 g in 50 ml of *water*, add 10 ml of *brominated hydrochloric acid AsT*, allow to stand for five minutes, and remove the excess of bromine by the addition of *stannous chloride solution AsT*.

Succinylsulphathiazole : Treat 5 g for one hour on a water-bath with 50 ml of *water* and 5 ml of *dilute ammonia solution*, filter, and evaporate the filtrate to dryness; boil the residue with 1 g of *anhydrous sodium carbonate AsT* and 10 ml of *water*, and add dropwise, while boiling, *bromine solution AsT* until the mixture is yellow; add 5 ml of *hydrochloric acid AsT*, boil gently to expel the bulk of the bromine, and remove the excess with a few drops of *stannous chloride solution AsT*; finally add 50 ml of *water* and 8 ml of *stannated hydrochloric acid AsT*.

Sulphafurazole : Treat 1 g as described under Aspirin.

Sulphamethazole : Treat 1 g as described under Aspirin.

Sulphaphenazole : Treat 5 g as described under Aspirin.

Tartaric acid : Treat 5 g as described under Aluminium Sulphate.

Titanium dioxide : Mix 1.25 g intimately with 2 g of *potassium carbonate* and ignite in a muffle furnace at about 950° for thirty minutes. Allow to cool, break up the mass transfer to a flask, add 0.1 ml of *stannous chloride AsT* and 30 ml of *hydrochloric acid (constant boiling*

composition) AsT, attach a condenser to the flask, heat at just below the boiling-point for one hour, and then distil 16 ml, to the distillate add 45 ml of *water* and 0.1 ml of *stannous chloride solution AsT*.

Tragacanth : Treat 3.3 g as described under Aspirin.

Dried yeast : Heat gently 5 g with 25 ml of *water* and 10 ml of *nitric acid AsT* in a long-necked flask until frothing has subsided. Cool, add gradually 10 ml of *sulphuric acid AsT*, and heat until the mixture begins to darken; then add *nitric acid AsT*, dropwise, and continue heating until white fumes are evolved and the liquid is almost colourless. Cool, add 25 ml of a saturated solution of *ammonium oxalate AsT* in *water*, and boil until white fumes are evolved; cool, dilute to 50 ml with *water*, and add 10 ml of *stannated hydrochloric acid AsT* and 0.1 ml of *stannous chloride solution AsT*.

Zinc oxide : Treat 1 g as described under Calcium Carbonate.

Zinc stearate : Treat 5 g as described under Sodium Salicylate.

Zinc sulphate : Treat 10 g as described under Aluminium Sulphate.

3.2.2 Limit Test for Chlorides

Dissolve the specified quantity of the substance in *water*, or prepare a solution as directed in the text and transfer to a *Nessler cylinder*. Add 10 ml of *dilute nitric acid*, except when nitric acid is used in the preparation of the solution, dilute to 50 ml with *water*, and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for 5 minutes. The opalescence produced is not greater than the *standard opalescence*, when viewed transversely.

Standard Opalescence

Place 1.0 ml of a 0.05845 per cent w/v solution of *sodium chloride* and 10 ml of *dilute nitric acid* in a *Nessler cylinder*. Dilute to 50 ml with *water* and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for five minutes.

3.2.3 Test for Free Formaldehyde

Use either of the following methods: Method A is unsuitable when metabisulphite has been used to neutralise excess formaldehyde in the vaccine.

Method A

Standard formaldehyde solution: Dilute a suitable volume of *formaldehyde solution* with *water* to give a solution containing 20 µg of formaldehyde, CH_2O per ml.

Procedure : To 1.0 ml of a 1 in 10 dilution of the preparation to be examined in a test-tube, add 4.0 ml of *water* and 5.0 ml of *acetylacetone solution*. Place the tube in a water-bath at 40° for forty minutes. The solution is not more intensely coloured than a reference solution prepared at the same time and in the same manner using 1.0 ml of *standard formaldehyde solution* in place of the dilution of the preparation being examined. The comparison should be made examining the tubes down their vertical axes.

Method B

Standard formaldehyde solution : Prepare as directed under Method A.

Magenta-sulphite solution : Dissolve 0.1 g of *basic magenta* with slight warming in 60 ml of *water*. Cool and dissolve in the solution 2 g of *anhydrous sodium sulphite*. Slowly add 1.0 ml of *hydrochloric acid* with continuous shaking, dilute with *water* to 100 ml, and filter. The solution, which is normally coloured orange, may be used immediately.

Procedure : Dilute 1.0 ml of the preparation being examined, to 10.0 ml with *water* and centrifuge. Transfer 2.0 ml of the supernatant liquid to a *Nessler cylinder*, add 20 ml of *water*, 10 ml of a 50 per cent v/v solution of *sulphuric acid*, 10 ml of *magenta-sulphite solution*, and sufficient *water* to produce 50 ml. Allow to stand for sixty minutes. The solution is not more intensely coloured than a reference solution prepared at the same time and in the same manner using 2.0 ml of *standard formaldehyde solution* in place of the dilution of the preparation being examined.

3.2.4 Limit Test For Heavy Metals

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced be the substance with that of a control prepared from a standard lead solution.

Determine the amount of heavy metals by one of the following methods and as directed in the individual monograph: Method A is used for substances that yield clear, colourless solutions under the specified test conditions. Method B is used for substances that do not yield clear, colourless solutions under the test conditions specified for Method A, or for substances which, by virtue of their complex nature, interfere with the precipitation of metals by sulphide ion. Method C is used for substances that yield clear, colourless solutions with *sodium hydroxide solution*.

Special Reagents

Acetic acid Sp. : *Acetic acid* which complies with the following additional test: Make 25 ml alkaline with *dilute ammonia solution Sp.*, add 1 ml of *potassium cyanide*

solution Sp., dilute to 50 ml with *water* and add two drops of *sodium sulphide solution*; no darkening is produced.

Dilute acetic acid Sp. : *Dilute acetic acid* which complies with the following additional test: Evaporate 20 ml in a porcelain dish, nearly to dryness on a water-bath. Add to the residue 2 ml of the acid and dilute with *water* to 25 ml, add 10 ml of *hydrogen sulphide solution*. Any dark colour produced is not more than that of a control solution consisting of 2 ml of the acid and 4.0 ml of *standard lead solution* diluted to 25 ml with *water*.

Ammonia solution Sp. : *Strong ammonia solution* which complies with the following additional test : Evaporate 10 ml to dryness on a water-bath; to the residue add 1 ml of *dilute hydrochloric acid Sp.* and evaporate to dryness. Dissolve the residue in 2 ml of *dilute acetic acid Sp* and sufficient *water* to produce 25 ml, add 10 ml of *hydrogen sulphide solution*. Any darkening produced is not greater than in a blank solution containing 2 ml of *dilute acetic acid Sp* 1.0 ml of *standard lead solution* and sufficient *water* to produce 25 ml.

Dilute ammonia solution Sp. : *Dilute ammonia solution* which complies with the following additional test: To 20 ml add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water*, and add two drops of *sodium sulphide solution*; no darkening is produced.

Hydrochloric acid Sp. : *Hydrochloric acid* which complies with the following additional test: Evaporate 17 ml of the acid in a beaker to dryness on a water-bath. Dissolve the residue in 2 ml of *dilute acetic acid Sp.*, dilute to 40 ml with *water* and add 10 ml of *hydrogen sulphide solution*; any darkening produced is not greater than in a blank solution containing 2.0 ml of *standard lead solution*, 2 ml of *dilute acetic acid Sp.*, and diluted to 40 ml with *water*.

Dilute hydrochloric acid Sp. : *Dilute hydrochloric acid*, which complies with the following additional test : Treat 19 ml of the acid in the manner described under *Hydrochloric acid Sp.*.

Lead nitrate stock solution : Dissolve 0.1598 g of *lead nitrate* in 100 ml of *water* to which has been added 1 ml of nitric acid, then dilute with *water* to 1000.0 ml.

This solution must be prepared and stored in polyethylene or glass containers free from soluble lead salts.

Standard lead solution : On the day of use, dilute 10.0 ml of *lead nitrate stock solution* with *water* to 100.0 ml. Each ml of *standard lead solution* contains the equivalent of 10 µg of lead. A control comparison solution prepared with 2.0 ml of *standard lead solution* contains, when compared to a solution representing 1.0 g of the substance being tested, the equivalent of 20 parts per million of lead.

Nitric acid Sp. : *Nitric acid* which complies with the following additional test : Dilute 10 ml with 10 ml of *water*, make alkaline with *ammonia solution Sp.*, add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water*, and add two drops of *sodium sulphide solution* ; no darkening is produced.

Potassium cyanide solution Sp. : *See* Appendix 3.2.6.

Sulphuric acid Sp. : *Sulphuric acid* which complies with the following additional test : Add 5 g to 20 ml of *water*, make alkaline with *ammonia solution Sp.*, add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water* and add two drops of *sodium sulphide solution* ; no darkening is produced.

Method A

Standard solution : Into a 50-ml *Nessler cylinder*, pipette 2 ml of *standard lead solution* and dilute with *water* to 25 ml. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp.* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml, and mix.

Test solution : Into a 50-ml *Nessler cylinder*, place 25 ml of the solution prepared for the test as directed in the individual monograph; or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with *water* to 25 ml the specified quantity of the substance being tested. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp.* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Procedure : To each of the cylinders containing the *standard solution* and *test solution* respectively, add 10 ml of freshly prepared *hydrogen sulphide solution*, mix, dilute with *water* to 50 ml, allow to stand for five minutes, and view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution*.

Method B

Standard solution : Proceed as directed under Method A.

Test solution : Weigh in a suitable crucible the quantity of the substance specified in the individual monograph, add sufficient *sulphuric acid Sp.* to wet the sample, and ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of *nitric acid Sp.* and five drops of *sulphuric acid Sp.*, and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burnt off. Cool, add 4 ml of *hydrochloric acid Sp.*, cover, digest on a water-bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with one drop of *hydrochloric acid Sp.*, add 10 ml of hot *water* and digest for two minutes. Add *ammonia solution Sp.*, dropwise, until the solution is just alkaline to *litmus paper*, dilute with *water* to 25 ml and adjust with *dilute acetic acid Sp.* to a pH between 3.0 and 4.0. Filter if necessary, rinse the crucible and the filter with 10 ml of *water*, combine the filtrate and washings in

a 50 ml *Nessler cylinder*, dilute with *water*, to about 35 ml, and mix.

Procedure : Proceed as directed under Method A.

Method C

Standard solution : Into a 50-ml *Nessler cylinder*, pipette 2 ml of *standard lead solution*, add 5 ml of *dilute sodium hydroxide solution*, dilute with *water* to 50 ml and mix.

Test solution : Into a 50-ml *Nessler cylinder*, place 25 ml of the solution prepared for the test as directed in the individual monograph; or, if not specified otherwise in the individual monograph, dissolve the specified quantity in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution*. Dilute to 50 ml with *water* and mix.

Procedure : To each of the cylinders containing the *standard solution* and the *test solution*, respectively, add 5 drops of *sodium sulphide solution*, mix, allow to stand for five minutes and view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution*.

3.2.5 Limit Test For Iron

Standard iron solution : Weigh accurately 0.1726 g of *ferric ammonium sulphate* and dissolve in 10 ml of *0.1 N sulphuric acid* and sufficient *water* to produce 1000.0 ml. Each ml of this solution contains 0.02 mg of Fe.

Method

Dissolve the specified quantity of the substance being examined, in 40 ml of *water*, or use 10 ml of the solution prescribed in the monograph, and transfer to a *Nessler cylinder*. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, dilute to 50 ml with *water* and allow to stand for five minutes. Any colour produced is not more intense than the standard colour.

Standard colour : Dilute 2.0 ml of *standard iron solution* with 40 ml of *water* in a *Nessler cylinder*. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, dilute to 50 ml with *water* and allow to stand for five minutes.

3.2.6 Limit Test for Lead

The following method is based on the extraction of lead by solutions of dithizone. All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm *dilute nitric acid*, followed by *water*.

Special Reagents

(1) Ammonia-cyanide solution Sp. : Dissolve 2 g of *potassium cyanide* in 15 ml of *strong ammonia solution* and dilute with *water* to 100 ml.

(2) Ammonium citrate solution Sp. : Dissolve 40 g of *citric acid* in 90 ml of *water*. Add two drops of *phenol red solution* then add slowly *strong ammonia solution* until the solution acquires a reddish colour. Remove any lead present by extracting the solution with 20-ml quantities of *dithizone extraction solution* until the dithizone solution retains its orange-green colour.

(3) Dilute standard lead solution : Dilute 10.0 ml of *standard lead solution* with sufficient 1 per cent v/v solution of *nitric acid* to produce 100.0 ml. Each ml of this solution contains 1 µg of lead per ml.

(4) Dithizone extraction solution : Dissolve 30 mg of *diphenylthiocarbazone* in 1000 ml of *chloroform*, and add 5 ml of *alcohol*. Store the solution in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1 per cent v/v solution of *nitric acid* and discard the acid.

(5) Hydroxylamine hydrochloride solution Sp. : Dissolve 20 g of *hydroxylamine hydrochloride* in sufficient *water* to produce about 65 ml. Transfer to a separator, add five drops of *thymol blue solution*, add *strong ammonia solution* until the solution becomes yellow. Add 10 ml of a 4 per cent w/v solution of *sodium diethyldithiocarbamate* and allow to stand for five minutes. Extract with successive quantities, each of 10 ml, of *chloroform* until a 5 ml portion of the extract does not assume a yellow colour when shaken with *dilute copper sulphate solution*. Add *dilute hydrochloric acid* until the solution is pink and then dilute with sufficient *water* to produce 100 ml.

(6) Potassium cyanide solution Sp. : Dissolve 50 g of *potassium cyanide* in sufficient *water* to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml, of *dithizone extraction solution* until the dithizone solution retains its orange-green colour. Extract any dithizone remaining in the cyanide solution by shaking with *chloroform*. Dilute this cyanide solution with sufficient *water* to produce a solution containing 10 g of potassium cyanide in each 100 ml.

(7) Standard dithizone solution : Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of *chloroform*. Store the solution in a glass-stoppered, lead-free bottle, protected from light and in a refrigerator.

(8) Citrate-cyanide wash solution : To 50 ml of *water* add 50 ml of *ammonium citrate solution Sp.* and 4 ml of *potassium cyanide solution Sp.*, mix, and adjust the pH, if necessary, with *strong ammonia solution* to 9.0.

(9) Buffer solution pH 2.5 : To 25.0 ml of *0.2 M potassium hydrogen phthalate* add 37.0 ml of *0.1 N hydro-*

chloric acid, and dilute with sufficient *water* to produce 100.0 ml.

(10) Dithizone-carbon tetrachloride solution : Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of *carbon tetrachloride*. Prepare this solution fresh for each determination.

(11) pH 2.5 wash solution : To 500 ml of a 1 per cent v/v *nitric acid* add *strong ammonia solution* until the pH of the mixture is 2.5, then add 10 ml of *buffer solution pH 2.5* and mix.

(12) Ammonia-cyanide wash solution : To 35 ml of *pH 2.5 wash solution* add 4 ml of *ammonia-cyanide solution Sp.*, and mix.

Method

Transfer the volume of the prepared sample directed in the monograph to a separator, and unless otherwise directed in monograph, add 6 ml of *ammonium citrate solution Sp.*, and 2 ml of *hydroxylamine hydrochloride solution Sp.* (For the determination of lead in iron salts use 10 ml of *ammonium citrate solution Sp.*). Add two drops of *phenol red solution* and make the solution just alkaline (red in colour) by the addition of *strong ammonia solution*. Cool the solution if necessary, and add 2 ml of *potassium cyanide solution Sp.* Immediately extract the solution with several quantities, each of 5 ml, of *dithizone extraction solution*, draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combined dithizone solutions for 30 seconds with 30 ml of a 1 per cent w/v solution of *nitric acid* and discard the chloroform layer. Add to the acid solution exactly 5 ml of *standard dithizone solution* and 4 ml of *ammonia-cyanide solution Sp.* and shake for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *dilute standard lead solution* equivalent to the amount of lead permitted in the sample under examination.

3.2.7 Sulphated Ash

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid*, heat gently until white fumes are no longer evolved and ignite at 800° ± 25° until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighings do not differ by more than 0.5 mg.

3.2.8 Limit Test for Sulphates

Reagents

Barium sulphate reagent : Mix 15 ml of *0.5M barium chloride*, 55 ml of *water*, and 20 ml of *sulphate-free alcohol*, add 5 ml of a 0.0181 per cent w/v solution of *potassium sulphate*, dilute to 100 ml with *water*, and mix. Barium Sulphate Reagent must be freshly prepared.

0.5 M Barium chloride : *Barium chloride* dissolved in *water* to contain in 1000 ml 122.1 g of $BaCl_2,2H_2O$.

Method

Dissolve the specified quantity of the substance in *water*, or prepare a solution as directed in the text, transfer to a *Nessler cylinder*, and add 2 ml of *dilute hydrochloric acid*, except where hydrochloric acid is used in the preparation of the solution. Dilute to 45 ml with *water*, add 5 ml of *barium sulphate reagent*, stir immediately with a glass rod, and allow to stand for five minutes. The turbidity produced is not greater than the *standard turbidity*, when viewed transversely.

Standard turbidity : Place 1.0 ml of a 0.1089 per cent w/v solution of *potassium sulphate* and 2 ml of *dilute hydrochloric acid* in a *Nessler cylinder*, dilute to 45 ml with *water*, add 5 ml of *barium sulphate reagent*, stir immediately with a glass rod, and allow to stand for five minutes.

3.3. OTHER TESTS AND ASSAYS

3.3.1 Determination of Aluminium in Adsorbed Vaccines

Shake the sample thoroughly and transfer a suitable volume, equivalent to about 5 mg of aluminium, to a 50-ml combustion flask. Add 1 ml of *sulphuric acid*, 0.1 ml of *nitric acid* and a few glass beads or a little pumice powder. Heat the solution until thick, white fumes are evolved. If any charring takes place add a few more drops of *nitric acid* and continue boiling until the solution is colourless. Allow to cool for a few minutes, carefully add 10 ml of *water* and boil until a clear solution is obtained. Allow to cool, add 0.05 ml of *methyl orange solution* and neutralise with *sodium hydroxide solution*. If any precipitate separates, dissolve it by adding, dropwise, sufficient *dilute sulphuric acid*. Transfer to a 250-ml conical flask, rinse the combustion flask with small quantities of *water* and add to the main solution. Add 25.0 ml of *0.02M disodium ethylenediamine tetraacetate*, 10 ml of *buffer solution, pH 4.4* and boil gently for three minutes. Add 0.1 ml of *pyridylazonaphthol solution* and titrate the hot solution with *0.02M copper sulphate* until the colour changes to purplish-brown. Carry out a blank determination omitting the sample. Each ml of *0.02M disodium ethylenediamine tetraacetate* is equivalent to 0.0005396 g of Al.

3.3.2 Determination of Esters

Boil a convenient quantity of *alcohol* thoroughly to expel carbon dioxide and neutralise it to *phenolphthalein solu-*

tion. Unless otherwise stated in the monograph, weigh accurately about 2 g of the substance being examined or other suitable quantity, so that the volume of *0.5N alcoholic potassium hydroxide* to be added is at least twice that theoretically required, dissolve in 5 ml of the neutralised alcohol contained in a hard-glass flask, and neutralise the free acid in the solution with *0.1N alcoholic potassium hydroxide*, using 0.2 ml of *phenolphthalein solution* as indicator. Add 25.0 ml of *0.5N alcoholic potassium hydroxide*, attach the flask to a reflux condenser, and boil on a water-bath for one hour. Add 20 ml of *water*, and titrate the excess of alkali with *0.5N hydrochloric acid*, using a further 0.2 ml of *phenolphthalein solution* as indicator. Carry out the same operation without the substance under examination. The difference between the titrations is equivalent to the alkali required to saponify the esters.

3.3.3 Determination of Methoxyl

Apparatus

The apparatus consists of a 50-ml round-bottomed boiling-flask into which is sealed a capillary side arm of 1 mm diameter to provide an inlet for a stream of *carbon dioxide* or *nitrogen*. The flask is also fitted with an upright air condenser about 25 cm long and about 9 mm in diameter, bent through 180° at the top and terminating in a glass capillary of 2 mm diameter dipping into a small scrubber containing about 2 ml of *water*. The outlet from the scrubber is a tube of about 7 mm diameter terminating in a removable tube of 4 mm diameter which dips below the surface of the liquid in the first of two receivers connected in series.

Method

Weigh accurately a quantity of the substance being examined, approximately equivalent to 50 mg of methyl iodide, and place it in the boiling-flask. Add a boiling-rod, 2.5 ml of melted *phenol* and 5 ml of *hydroiodic acid* and connect the flask with the remainder of the apparatus. The first receiver contains about 6 ml and the second receiver about 4 ml of a 10 per cent w/v solution of *potassium acetate* in *glacial acetic acid* to which 0.2 ml of *bromine* has been added. Pass a slow uniform stream of *carbon dioxide* or *nitrogen* through the side arm of the boiling-flask, and gently heat the liquid by means of a mantled micro-burner at such a rate that the vapours of the boiling liquid rise halfway up the condenser. For most substances thirty minutes is sufficient to complete the reaction and sweep out the apparatus. Wash the contents of both receivers into a 250-ml glass-stoppered conical flask containing 5 ml of a 25 per cent w/v solution of *sodium acetate*, adjust the volume of the liquid to approximately 125 ml, and add 0.3 ml of *formic acid*. Rotate the flask until the colour due to the bromine is discharged, add 0.5 ml of *formic acid*, stopper the flask and mix the contents thoroughly so as to remove any excess of bromine from the vapour above the liquid in the flask; allow to stand for one to two minutes. Add 1 g of *potassium iodide* and a few ml of *N sulphuric acid* and titrate the liberated iodine with *0.1N sodium thiosulphate*. Repeat the operation without the substance being examined and deduct the volume of *0.1N sodium thiosulphate* required from the volume required in the determination of methoxyl. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.0005172 g of methoxyl (CH_3O).

3.3.4 Nitrite Titration

The following method is suitable for the determination of most of the pharmacopoeial sulphonamide drugs and their preparations. It may also be used for other pharmacopoeial drugs for which nitrite titration is recommended.

Apparatus

A suitable open vessel, of about 200 ml capacity is fitted with two similar clean platinum electrodes and a stirrer. The electrodes may be of platinum foil 0.5 cm square and should be placed 1.5 cm apart. They may be cleaned by immersing for a few seconds in boiling *nitric acid* containing a small amount of *ferric chloride*, followed by washing with *water*.

The polarising voltage may be obtained from a 1.5-volt dry cell and potentiometer or other convenient device which enables a small but definite voltage to be applied across the electrodes. The current flowing in the system is indicated by a series galvanometer, which should have adequate sensitivity.

Procedure

Weigh accurately about 0.5 g in the case of a sulphonamide or otherwise the quantity specified in the individual monograph and transfer to the titration vessel. Add 20 ml of *hydrochloric acid* and 50 ml of *water*, stir until dissolved, cool to about 15°. Immerse the platinum electrodes in the solution and apply a voltage of about 50 mV across the electrodes, when polarisation of the electrodes takes place. Place the burette tip just above the surface of the solution and stir the solution gently, maintaining the temperature at about 15°. Add *0.1M sodium nitrite* slowly and when the titration is within 1 ml of the end-point, add the titrant in 0.1 ml portions, allowing not less than 1 minute between additions (The galvanometer needle deflects and then returns to approximately its original position until the end-point is reached). At the end-point, when a slight excess of sodium nitrite is present, the electrodes are depolarised, current flows and a permanent deflection of the needle is obtained.

NOTE – It will be necessary to adjust the sensitivity of the galvanometer or the applied voltage before the titration is begun in order to obtain an adequate deflection at the end-point.

3.3.5 Determination of Nitrogen

The following methods are suitable for the determination of nitrogen in most substances but there are some alkaloids and other nitrogen containing organic compounds which do not yield all of their nitrogen upon digestion with sulphuric acid; therefore these methods cannot be used in all organic compounds.

Method A

Weigh accurately the quantity of the substance being examined, specified in the monograph or a quantity equivalent to about 35 mg of nitrogen, into a 200-ml long-necked flask and add 3 g of *anhydrous sodium sulphate*, 0.3 g of *nitrogen-free mercuric oxide*, and 20 ml of *nitrogen-free sulphuric acid*, unless otherwise specified in the monograph. Heat the mixture over a small flame until colourless and boil gently for a further two hours, unless otherwise directed in the monograph; care should be taken to prevent the upper part of the flask from becoming over heated. Cool, dilute to about 75 ml with *water* and add a piece of *granulated zinc* and a solution containing 1.5 g of *sodium hydroxide* per ml of *sulphuric acid* used and 2 g of *sodium thiosulphate* in 25 ml of *water*. Ensure that before distillation, the mixture is strongly alkaline by increasing, if necessary, the quantity of *sodium hydroxide*. Immediately connect the flask to a distillation apparatus, mix the contents, distil the liberated ammonia into 50.0 ml of *0.1N sulphuric acid* and titrate the excess of acid with *0.1N sodium hydroxide*, using *methyl red-methylene blue solution* as indicator. Repeat the operation without the substance being examined; the difference between the titrations represents the ammonia liberated by the substance being examined. Each ml of *0.1N sulphuric acid* is equivalent to 0.001401 g of N.

Method B

Weigh accurately the quantity of the substance being examined, specified in the monograph or a quantity equivalent to about 35 mg of nitrogen, into a 200-ml long-necked flask, add 20 ml of *nitrogen-free sulphuric acid*, unless otherwise specified in the monograph, and heat for fifteen minutes. Add 3 g of *anhydrous sodium sulphate* and 0.3 g of *nitrogen-free mercuric oxide* and complete Method A, beginning at the words "Heat the mixture. . . .".

Method C

Weigh accurately the quantity of the substance being examined, specified in the monograph or a quantity equivalent to about 15 mg of nitrogen, into a 200-ml long-necked flask, and add 1 g of a powdered mixture of 10 parts of *anhydrous sodium sulphate* or *potassium sulphate* and 1 part of *copper sulphate*. Add 10 ml of *nitrogen-free sulphuric acid*, mix, and carefully add 1 ml of *hydrogen peroxide solution (30 per cent)* down the wall of the flask. Heat until the solution becomes clear green in colour or almost colourless for thirty minutes. Cool, carefully add 20 ml of *water*, cool again and connect the flask to a distillation apparatus. Add 50 ml of *10M sodium hydroxide* and distil immediately by passing steam through the flask. Collect the distillate in 25.0 ml of *0.1N hydrochloric acid* and titrate the excess of acid with *0.1N sodium hydroxide*, using *methyl red-methylene blue solution* as indicator. Repeat the operation using 0.025 g of *dextrose (anhydrous)* in place of the substance being examined; the difference between the titration represents the ammonia liberated by the substance being examined. Each ml of *0.1N hydrochloric acid* is equivalent to 0.001401 g of N.

Method D : (When nitrates and nitrites are present)

Weigh accurately the quantity of the substance being examined, specified in the monograph or a quantity equivalent to about 15 mg of nitrogen, into a 200-ml long-necked flask, and add 10 ml of *nitrogen-free sulphuric acid* in which 0.2 g of *salicylic acid* has been previously dissolved and mix. Allow the mixture to stand for thirty minutes with frequent shaking. To the mixture add 10 parts of *anhydrous sodium sulphate* or *potassium sulphate* and 1 part of *copper sulphate* and 1 ml of *hydrogen peroxide solution (30 per cent)* poured carefully down the wall of the flask. Complete Method C, beginning at the words "Heat until the solution . . . . .".

Method E

Apparatus : A unit of the type generally known as semi-micro Kjeldahl apparatus.

Method

Weigh accurately a quantity of the substance being examined equivalent to about 2 mg of nitrogen into the digestion flask of the apparatus. Add 1 g of a powdered mixture of 10 parts of *anhydrous sodium sulphate* or *potassium sulphate* and 1 part of *copper sulphate* and wash down any adhering material from the neck of the flask with *water*. Add 7 ml of *nitrogen-free sulphuric acid* and 1 ml of *hydrogen peroxide solution (30 per cent)* carefully down the well of the flask (Do not add hydrogen peroxide during the digestion). Heat until the solution has a clear blue colour and the sides of the flask are free from carbonaceous matter. Cool, add carefully 20 ml of *water*, cool the solution and arrange for steam distillation. Add, through the funnel 30 ml of a 40 per cent w/v solution of *sodium hydroxide*, rinse the funnel with 10 ml of *water*, tightly close the apparatus, and begin the distillation with steam immediately. Collect the distillate in 25.0 ml of *0.01N sulphuric acid*, continue the distillation until the distillate measures about 100 ml. Titrate the distillate with *0.01N sodium hydroxide* using *methyl red-methylene blue solution* as indicator. Perform a blank determination; the difference between the titration represents the ammonia liberated by the substance being examined. Each ml of *0.01N sulphuric acid* is equivalent to 0.0001401 g of N.

3.3.6 Oxygen-Flask Method

CAUTIONARY NOTE – Great care must be taken in carrying out the following method. It is advisable to wear safety glasses and to use a suitable safety screen particularly when combustion takes place.

Apparatus

The apparatus consists of a thick-walled, conical 500-ml iodine flask, fitted with a ground-glass stopper to which is fused a piece of platinum wire about 13 cm long and 1 mm in diameter and to which is attached a piece of platinum gauze to serve as means of holding the sample. The gauze may be about 2 cm wide and 1.5 cm long and should comply with the dimensions of a No. 36 sieve. The flask must be well-cleaned and free from even traces of organic solvents.

Method

Solid substances should be finely ground and thoroughly mixed before the specified quantity is weighed.

For liquids place the specified quantity on about 15 mg of ashless filter-paper flock contained in one part of a methyl cellulose capsule of a suitable size, close the capsule, inserting one end of a narrow strip of filter-paper between the two parts, and secure the capsule in the platinum gauze. Ointments should be enclosed in grease-proof paper before wrapping in filter paper.

Weigh accurately a suitable quantity of the substance being examined and wrap, if a solid in a piece of halide-free filter paper about 5 cm long and 3 cm wide, secure the package in the platinum gauze sample holder, and insert one end of a narrow strip of filter paper in the roll to serve as a fuse. Flush the flask with *oxygen*, moisten the neck with *water*, place the specified absorbing liquid in the flask, fill it with *oxygen* by swirling the liquid to favour its taking up oxygen light the free end of the fuse-strip, and immediately insert the stopper. Hold the stopper firmly in place when vigorous burning has begun, invert the flask so as to provide a liquid seal but taking care to prevent incompletely burned material falling into the liquid. When combustion is complete shake the flask vigorously for about five minutes, place a few ml of *water* in the cup top, carefully remove the stopper, and rinse the stopper, platinum wire, platinum gauze, and sides of the flask with *water*. Proceed as directed in the following methods:

For bromine : Burn the specified quantity of the substance being examined in the prescribed manner using as the absorbing liquid 15 ml of a mixture of 1 volume of *hydrogen peroxide solution (30 per cent)* and 9 volumes of *N sulphuric acid*. When the process is complete, cool in ice for fifteen minutes, add 5 ml of *2N nitric acid* and 10 ml of *0.1N silver nitrate* and titrate with *0.05N ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator and shaking vigorously as the end point is approached. Repeat the operation omitting the substance being examined. The difference between the titrations represents the number of ml of *0.05N silver nitrate* required. Each ml of *0.05N silver nitrate* is equivalent to 0.003995 g of Br.

For chlorine : Burn the specified quantity of the substance being examined in the prescribed manner using 20 ml of *N sodium hydroxide* as the absorbing liquid. When the process is complete, add 2.5 ml of *nitric acid*, 2.5 ml of *water*, and 10 ml of *0.1N silver nitrate* and titrate with *0.05N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator and shaking vigorously as the end-point is approached. Repeat the operation omitting the substance being examined. The difference between the titrations represents the number of ml of *0.05N silver nitrate* required. Each ml of *0.05N silver nitrate* is equivalent to 0.001773 g of Cl.

For fluorine : Burn the specified quantity of the substance being examined in the prescribed manner using 20 ml of *water* as the absorbing liquid. When the process is complete, add sufficient *water* to produce 50.0 ml. To 2.0 ml add 20 ml of *water*, 10 ml of *alizarine fluorine blue solution*, 3 ml of a solution containing 12 per cent w/v of *sodium acetate* and 6 per cent v/v of *glacial acetic acid*, 10 ml of *cerous nitrate solution*, and sufficient *water* to produce 50.0 ml. Allow to stand in the dark for one hour and measure the *extinction* of a 1-cm layer of the resulting solution at 610 nm, Appendix 5.15 A, using as the blank a solution prepared as described above, beginning at the words "To 2.0 ml . . . " but using 2.0 ml of *water* instead of the test solution. Calculate the fluorine content from a reference curve prepared by treating suitable aliquots of a solution of *sodium fluoride* in the manner described above, beginning at the words "add 50 ml of *water*. . . . . ".

For iodine : Burn the specified quantity of the substance being examined in the prescribed manner using a mixture of 10 ml of *water* and 2 ml of *N sodium hydroxide* as the absorbing liquid. When the process is complete, add to the flask an excess (between 5 and 10 ml) of *acetic-bromine solution* and allow to stand for two minutes. Remove the excess of bromine by the addition of *formic acid* (about 0.5 to 1 ml), rinse the sides of the flask with *water*, and sweep out any bromine vapour above the liquid with a current of air. Add 1 g of *potassium iodide* and titrate with *0.02N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Each ml of *0.02N sodium thiosulphate* is equivalent to 0.000423 g of I.

For sulphur

Method I (in the absence of halogens and phosphorus) – Burn the specified quantity of the substance being examined in the prescribed manner, using 10 ml of *water* and 0.1 ml *hydrogen peroxide solution (30 per cent)* as the absorbing liquid. When the process is complete, cool the solution in ice for about fifteen minutes. Gently boil for two minutes, cool and add 50 ml of *ethanolic acetic-ammonia buffer pH 3.7*. Titrate with *0.05M barium perchlorate*, using 0.3 ml of *alizarin red S solution* as

indicator, until the solution becomes orange-pink in colour. Each ml of *0.05M barium perchlorate* is equivalent to 0.001603 g of S.

Method II (in the presence of halogens or phosphorus) – Burn the specified quantity of the substance being examined in the prescribed manner, using 15 ml of *water* and 1 ml of *hydrogen peroxide solution* as the absorbing liquid. When the process is complete, boil the solution for ten minutes, cool, and add 60 ml of *alcohol*. Titrate the solution with *0.01M barium perchlorate*, using 0.1 ml of 0.2 per cent w/v solution of *thoron* and 0.1 ml of a 0.0125 per cent w/v solution of *methylene blue* as indicator, until the yellow colour changes to pink. Each ml of *0.01M barium perchlorate* is equivalent to 0.0003206 g of S.

If the temperature at which the determinations prescribed under Methods I and II are performed differs from that at which the barium perchlorate solution was standardised, the titrant volumes are corrected by applying the expression:

$$V_c = V\,[1 + 0.0008\,(t_1 - t_2)],$$

where

V_c = the corrected volume of titrant,

V = the volume of titrant used,

t_1 = the temperature of the titrant during standardisation.

t_2 = the temperature of the titrant during the determination, and

3.3.7 Assay of Nitrous Oxide

Apparatus

The apparatus shown in the diagrams comprises a gas-burette of 100-ml capacity, connected through a two-way tap at its upper end to two capillaries, one of which (A) is used to introduce the gas into the apparatus, the other (B) being connected to a vertical capillary arm to form a four-way junction. The descending arm of the junction is connected to a condenser (C) of about 60-ml capacity, and the right arm of the junction is connected to a mercury manometer (M). Tap D on the upper vertical arm of the junction opens to the air. The lower part of the gas burette is fitted with a one-way tap connected by a rubber tube to a mercury reservoir. The upper part of the burette is graduated from 0 to 5 ml, and the lower part from 99.5 to 100.5 ml, both in increments of 0.1 ml (*see* Fig. 1).

Method

Close the three taps and immerse the condenser in liquid nitrogen, keeping the level slightly above the upper part of the condenser. By manipulating the two-way tap and the mobile reservoir create a partial vacuum in the apparatus, choosing an arbitrary pressure P between 6.7 and 8.0 kPa (50 to 60 torr), accurately measured. This pressure must remain constant for ten minutes to demonstrate that the apparatus is gas-tight.

Open the two-way tap to tube A and completely fill the burette and tube A with mercury. Close the two-way tap. Connect a rubber tube through a suitable pressure-relieving device to the exit valve of the cylinder of the gas being tested and pass a current of the gas through the rubber tube for one minute. Whilst the gas is still flowing, connect the rubber tube to the end of tube A and immediately open the two-way tap to tube A. Allow the specified volume of the gas to enter the burette by lowering the mercury reservoir. Close the two-way tap.

Raise the mercury reservoir slightly above the tube A and lower the level of the liquid nitrogen to the middle of the condenser. Carefully open the tap of the burette to connect with the condenser and allow the mercury to rise in the burette until it reaches the tap. Close the tap. Raise the level of the liquid nitrogen so as to totally immerse the condenser. Read the pressure and wait until it remains steady for two minutes.

Place the mercury reservoir in its bottom position and open the tap of the burette to make connection with the condenser. Move the mercury reservoir until the manometer reading is the same as the initial pressure P. Close the tap of the burette and, by means of the mercury reservoir, bring the pressure of the gas in the burette to atmospheric pressure. The number of ml of gas represents the non-condensible volume in the specified volume used.

After each series of 10 determinations allow atmospheric air to enter by opening the tap D, remove the liquid nitrogen from the condenser and allow the condenser to warm to room temperature.

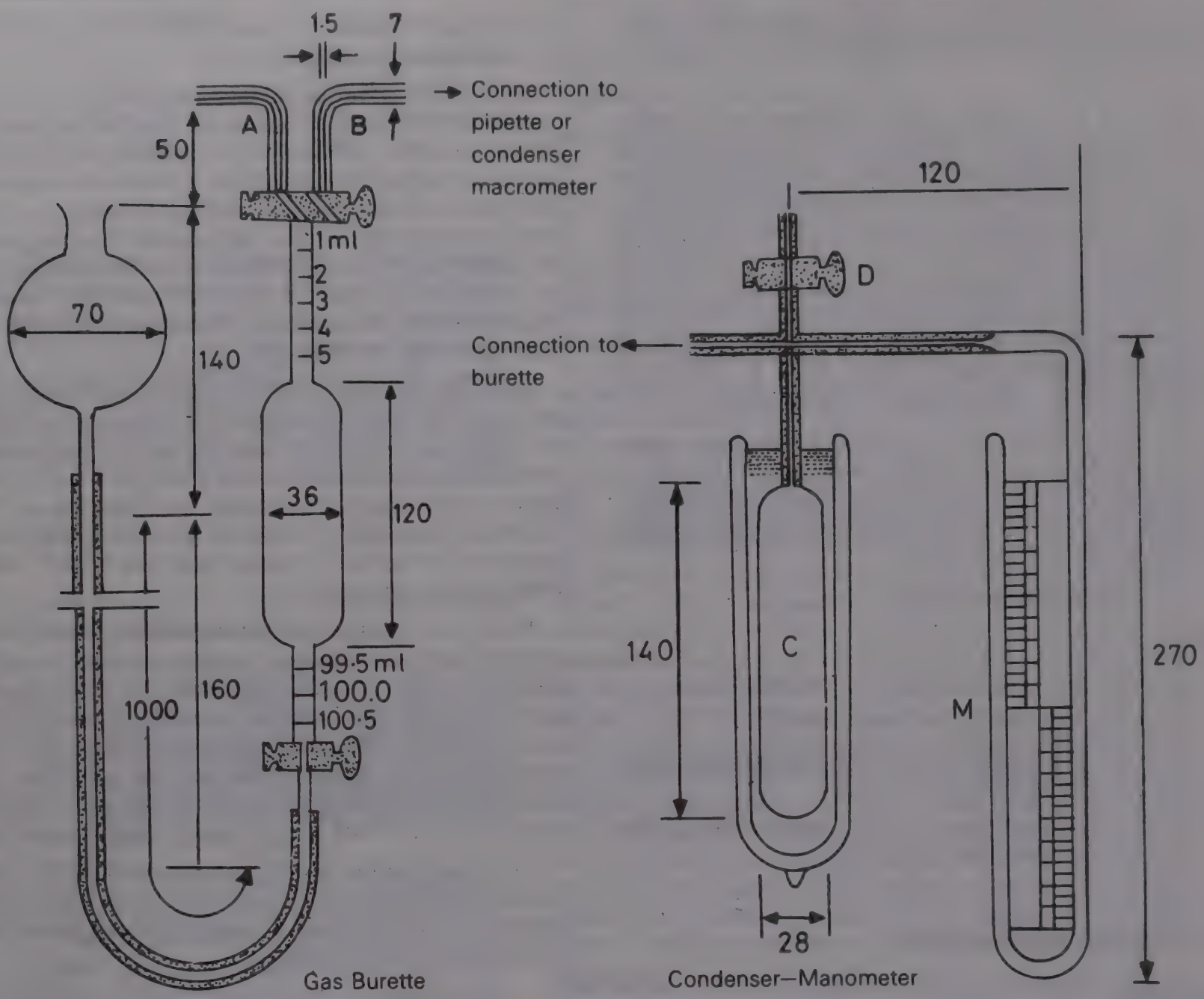
3.3.8 Assay of Oxygen

Apparatus

The apparatus comprises a gas-burette, of the type described under *assay of nitrous oxide*, in which tube B is connected to a gas-pipette comprising two bulbs of suitable size (*see* Fig. 2)

Method

Change the pipette with the reagents specified in the monograph. With the two-way tap open to tube B, draw the solution just to the level of the tap by moving the mercury reservoir. Open the two-way tap to tube A, completely fill the burette and tube A with mercury and close the two-way tap. Connect a rubber tube to the exit valve of the cylinder of the gas being tested through a suitable pressure reducing device and pass a current of the gas through the tube for one minute. Whilst the gas is still flowing, connect the rubber tube to tube A, immediately open the two-way tap to tube A, allow the specified quantity of the gas to enter the burette by lowering the mercury reservoir, and close the two-way tap. Increase the pressure



(Dimensions in mm unless otherwise stated)

Fig. 1 APPARATUS FOR THE ASSAY OF MEDICINAL GASES

of the gas by raising the mercury reservoir, open the two-way tap to tube B and transfer all the gas to the pipette. Close the tap and gently shake the pipette. After fifteen minutes, when most of the gas has been absorbed by the liquid, draw the residual gas back into the burette and repeat the procedure, beginning at the words 'Increase the pressure of the gas. . .', until the volume of residual gas is constant. Measure the volume of the residual gas in the burette.

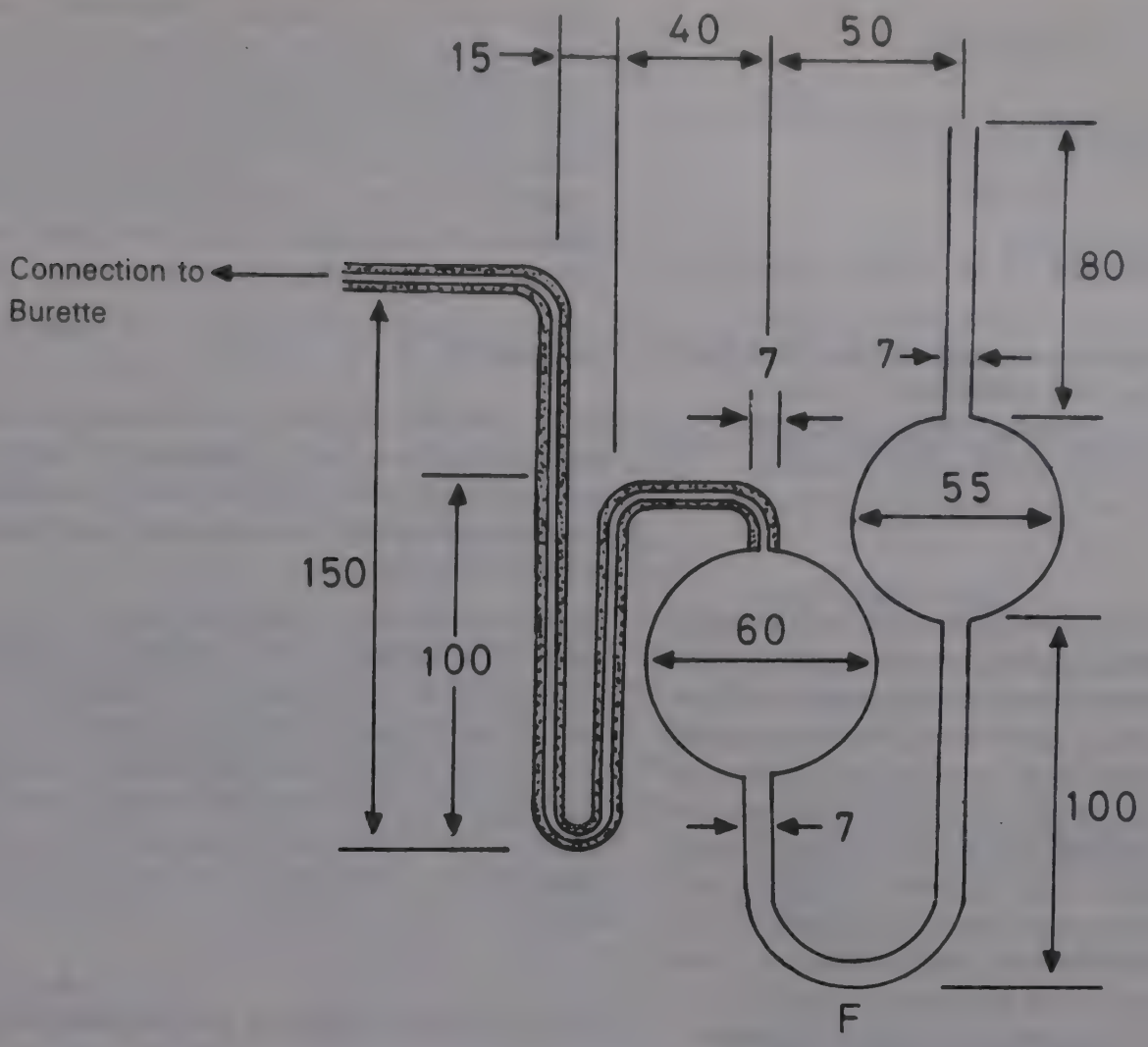
3.3.9 Determination of Phenol in Vaccines and Sera

Homogenise the preparation to be examined. Dilute an appropriate volume with *water* so as to obtain a solution expected to contain about 15 µg of phenol per ml. To 5.0 ml of this solution add 5 ml of *buffer solution pH 9.0*, 5 ml of *aminopyrazolone solution* and 5 ml of *potassium ferricyanide solution*. Allow to stand for ten minutes and then measure the *extinction* of a 1-cm layer of the resulting solution at the maximum at about 546 nm, Appendix 5.15 A. Calculate the phenol content from a calibration curve prepared from 5-ml quantities of a series of solutions of *phenol* containing 5 µg, 10µg, 15µg, 20 µg and 30 µg of phenol per ml, treated in the same manner.

3.3.10 Assay of Steroids

Standard Solution

Weigh accurately a suitable quantity of the *Reference substance* specified in the individual monograph, previously dried under the conditions specified in the individual monograph and dissolve in a suitable volume of *aldehyde-free ethyl alcohol*. Dilute quantitatively and stepwise with *aldehyde-free ethyl alcohol* to obtain a solution containing about 10 µg per ml. Pipette 20 ml of this solution into a glass-stoppered, 50-ml conical flask.



(Dimensions in mm unless otherwise stated)

Fig. 2 GAS PIPETTE

Test Solution

Prepare as directed in the individual monograph and pipette 20 ml into a glass-stoppered, 50-ml conical flask.

Method

To each of the flasks containing the Standard Solution and Test Solution, and to a similar flask containing 20.0 ml of *aldehyde-free ethyl alcohol* (the blank), add 2.0 ml of *blue tetrazolium solution* and mix. To each flask add 2.0 ml of a mixture of 1 volume of *tetramethyl-ammonium hydroxide solution (10 per cent)* and 9 volumes of *aldehyde-free ethyl alcohol* and mix and allow to stand in the dark for exactly 90 minutes at a temperature between 20° and 35°. Measure the *extinctions* of the solutions obtained from the Standard Solution and Test Solution at 525 nm against the blank, Appendix 5.15 A.

Calculation

The quantity in mg of steroid in the 20-ml aliquot of the Test Solution is given by the expression:

$$\frac{E_t}{E_s} \times C_s$$

in which E_t is the *extinction* of the Test Solution, E_s is the *extinction* of the Standard Solution and C_s is the quantity in mg of the Reference Substance in the 20-ml aliquot of the Standard Solution.

Calculate the quantity of steroid in the assay material on the basis of the aliquot of the Test Solution.

3.3.11 Identification of Steroids

Special Reagents

SOLVENTS

I. A mixture of one volume of *formamide* and nine volumes of *acetone*.

II. A mixture of one volume of *propyleneglycol* and nine volumes of *acetone*.

III. A mixture of one volume of *liquid paraffin* and nine volumes of *light petroleum (boiling range, 40° to 60°)*

MOBILE PHASES

A. *Chloroform*

B. A mixture of three volumes of *toluene* and one volume of *chloroform*.

C. *Toluene*

D. A mixture of four volumes of *cyclohexane* and one volume of *toluene*.

E. A mixture of equal volumes of *cyclohexane* and *light petroleum (boiling range, 40° to 60°)*.

F. A mixture of two volumes of *glacial acetic acid* and three volumes of *water*.

Method

Carry out the method for *thin-layer chromatography*, Appendix 5.4.3 using *kieselguhr G* as the coating substance. Place the dry plate in a tank containing a shallow layer of the specified solvent, allow the solvent to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use, with the flow of the mobile phase in the direction in which the aforementioned treatment was done, within two hours. Unless otherwise specified in the individual monograph, apply separately to the plate 2µl of each of two solutions in a mixture of nine volumes of *chloroform* and one volume of *methyl alcohol* containing (1) 0.25 per cent w/v of the substance under test, and (2) 0.25 per cent w/v of the appropriate Reference Standard, and at a third point, apply 2 µl of a mixture of equal volumes of solutions (1) and (2). Use the specified mobile phase. After removal of the plate allow the solvent to evaporate, heat at 120° for fifteen minutes and spray the hot plate with a 10 per cent v/v solution of *sulphuric acid* in *alcohol*. Heat at 120° for a further ten minutes, allow to cool and examine in daylight and under an ultra-violet lamp having a maximum output at about 366 nm. The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2). The principal spot in the third chromatogram appears as a single, compact spot.

3.3.12 Identification of Related Foreign Steroids

Method A

Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 77 volumes of *methylene chloride*, 15 volumes of *solvent ether*, 8 volumes of *methyl alcohol*, and 1.2 volumes of *water* as the mobile phase.

Apply separately to the plate 10 µl of each of three solutions in a mixture of 9 volumes of chloroform and 1 volume of *methyl alcohol* containing (1) 0.15 per cent w/v of the substance being examined, (2) 0.15 per cent w/v of the appropriate *Reference Substance (R.S.)*; (3) a 0.003 per cent w/v of each of *prednisolone R.S., prednisone, R.S.*, and *cortisone acetate R.S.* After removal of the plate, dry it in air until the solvents have evaporated, heat for ten minutes at 105°, cool and spray with *alkaline blue tetrazolium solution*. The principal spot in the chromatogram obtained with solution (1) corresponds in R value, colour and intensity to the principal spot obtained with solution (2). Any subsidiary spot in the chromatogram obtained with solution (1) is not more intense than the proximate spot obtained with solution (3).

Method B

Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and a mixture of 95 volumes of *ethylene chloride*, 5 volumes of *methyl alcohol*, and 0.2 volumes of *water* as the mobile phase.

Apply separately to the plate 10µl of each of three solutions in a mixture of 9 volumes of *chloroform* and 1 volume of *methyl alcohol* containing (1) 0.15 per cent w/v of the substance being examined; (2) 0.15 per cent w/v of the appropriate *Reference Substance* (R.S.); (3) 0.003 per cent w/v each of *prednisone R.S., prednisolone acetate R.S., cortisone acetate R.S.*, and *deoxycortone acetate R.S.* Complete the procedure described under Method A.

3.3.13 Determination of Sulphur Dioxide

Apparatus

A round-bottomed flask of 1000 to 1500 ml capacity is connected with a water-cooled reflux condenser whose upper end is connected with two absorption tubes in series. The flask in provided with a gas inlet tube which reaches nearly to the bottom of the flask. Each absorption tube contains 10 ml of *hydrogen peroxide solution* neutralised with *0.1N sodium hydroxide* using *bromophenol blue solution* as indicator.

Method

Place in the flask 500 ml of *water* and 20 ml of *hydrochloric acid*. Connect the flask with the condenser and absorption tubes, pass through it a steady current of *nitrogen* or *carbon dioxide* which has been bubbled through *sodium carbonate solution* and gradually heat the liquid until it boils. Maintain the current of *nitrogen* or *carbon dioxide*, allow the solution to boil for about ten minutes, and then cool the flask by gradual immersion in water. Introduce, by momentarily removing the stopper of the flask, 50 to 100 g of the substance being examined, heat gently, and boil for forty-five minutes. Turn off the current of *nitrogen* or *carbon dioxide*, disconnect the absorption tubes, and titrate the contents with *0.1N sodium hydroxide*. Each ml of *0.1N sodium hydroxide* is equivalent to 0.003203 g of sulphur dioxide.

Repeat the operation without the substance being tested; the solution in the absorption tubes remains neutral.

3.3.14 Acetyl Value

The acetyl value is the number which expresses in milligrams the amount of potassium hydroxide required to neutralise the acetic acid liberated by the hydrolysis of 1 g of the acetylated substance.

Method

1. Determine the *saponification value* of the substance to be examined, Appendix 3.3.20.

2. Acetylate the substance by the following method : Place 10 g with 20 ml of *acetic anhydride* in a long-necked, round-bottomed 200-ml flask, attached to a reflux air condenser. Support the flask on a sheet of asbestos in which a hole of about 4 cm in diameter has been cut and heat it with a small naked flame, not more than 25 mm in height, which does not impinge on the bottom of the flask. Boil gently for 2 hours, allow to cool, pour into 600 ml of *water* contained in a large beaker, add 0.2 g of pumice powder, and boil for thirty minutes. Cool, transfer to a separator and reject the lower layer. Wash the acetylated product with three or more quantities, each of 50 ml, of a warmed saturated solution of *sodium chloride* until the washings are no longer acid to *litmus paper*. Finally shake with 20 ml of warm *water* and remove the aqueous layer as completely as possible. Pour the acetylated substance into a small dish, add 1 g of powdered *anhydrous sodium sulphate*, stir thoroughly and filter through a dry pleated filter.

Determine the *saponification value* of the acetylated substance.

3. Calculate the Acetyl Value from the formula :

$$\text{Acetyl Value} = \frac{(b - a)\ 1335}{1335 - a}$$

where

a = Saponification Value of the substance

b = Saponification Value of the acetylated substance

3.3.15 Acid Value

The acid value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralise the free acids present in 1 g of substance.

Unless otherwise directed, dissolve about 10 g of the substance to be examined, accurately weighed, in 50 ml of a mixture of equal volumes of *alcohol* and *solvent ether*, previously neutralised with *0.1N sodium hydroxide* to *phenolphthalein*. If the sample does not dissolve in the cold solvent, connect the flask with a reflux condenser and warm slowly, with frequent shaking, until the sample dissolves. Add 1 ml of *phenolphthalein solution* and titrate with *0.1N sodium hydroxide* until the solution remains faintly pink after shaking for 30 seconds. Calculate the acid value from the following formula:

$$\text{Acid Value} = \frac{n \times 5.61}{w}$$

where

n = the number of ml of *0.1N* sodium hydroxide required

w = the weight in g of the substance

NOTE – If the oil has been saturated with carbon dioxide for the purpose of preservation, gently reflux the solution of the oil in alcohol and solvent ether for ten minutes before titration. The oil may be freed from the carbon dioxide by exposing it in a shallow dish in a vacuum desiccator for 24 hours before weighing the sample.

3.3.16 Ester Value

The ester value is the number of mg of potassium hydroxide required to saponify the esters present in 1 g of substance.

Determine the *acid value*, Appendix 3.3.15, of the substance being examined and the *saponification value*, Appendix 3.3.20. Calculate the ester value from the expression:

Ester Value = Saponification Value − Acid Value

3.3.17 Hydroxyl Value

The hydroxyl value is the number of mg of potassium hydroxide required to neutralise the acid combined by acetylation in 1.0 g of the substance.

Pyridine - Acetic Anhydride Reagent

Just before use, add cautiously with cooling 25 ml of freshly distilled *acetic anhydride* to 50 ml of freshly distilled *anhydrous pyridine*, cool and dilute with freshly distilled *anhydrous pyridine* to 100 ml.

Method

Unless otherwise indicated in the individual monograph, weigh accurately the quantity of the substance being examined, stated in the Table 1, in a 150-ml acetylation flask fitted with a condenser, and add the quantity of *pyridine-acetic anhydride reagent* stated in the Table 1. Boil for one hour on a water-bath, adjusting the level of the water to maintain it 2 to 3 cm above the level of the liquid in the flask. Cool, add 5 ml of *water* through the top of the condenser; if this causes cloudiness, add sufficient *pyridine* to produce a clear liquid. Shake, replace in the water-bath for ten minutes, remove and cool. Rinse the condenser and walls of the flask with 5 ml of *alcohol*, previously neutralised to 0.10 to 0.15 ml of *dilute phenolphthalein solution*. Titrate with *0.5N alcoholic potassium hydroxide*. Repeat the operation without the substance being examined.

TABLE 1

Presumed Hydroxyl Value	Quantity of Substance (g)	Volume of Pyridine-Acetic Anhydride Reagent (ml)
10 to 100	2.0	5.00
100 to 150	1.5	5.00
150 to 200	1.0	5.00
200 to 250	0.75	5.00
250 to 300	0.60 or 1.20	5.00 or 10.00
300 to 350	1.00	10.00
350 to 700	0.75	15.00
700 to 950	0.5	15.00

Calculate the hydroxyl value from the expression:

$$\text{Hydroxyl Value} = \frac{V \times 28.05}{w} + \text{Acid Value}$$

where

V = difference between the titrations.

w = weight in g of the substance

3.3.18 Iodine Value

The iodine value is the number which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100 g of the substance under the described conditions. It may be determined by any of the following methods:

Mehtod A

Place an accurately weighed quantity of the substance to be examined, in a dry 500-ml iodine flask, add 10 ml of *carbon tetrachloride*, and dissolve. Add 20 ml of *iodine monochloride solution*, insert the stopper, previously moistened with *potassium iodide solution*, and allow to stand in a dark place at a temperature between 15° and 25° for 30 minutes. Place 15 ml of *potassium iodide solution* in the cup top, carefully remove the stopper, rinse the stopper and the sides of the flask with 100 ml of *water*, shake, and titrate with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being examined, and note the number of ml of *0.1N sodium thiosulphate* required (b)

Calculate the iodine value from the formula:

$$\text{Iodine Value} = \frac{(b-a) \times 1.269}{w}$$

where

w = weight in g of the substance

The approximate weight, in g, of substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogan is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Method B

Place an accurately weighed quantity of the substance to be examined, in a 300-ml iodine flask fitted with a ground glass stopper which is dry or has been rinsed with *glacial acetic acid*, and add 15 ml of *chloroform* to dissolve. Add slowly from a burette 25.0 ml of *iodine bromide solution*, insert the stopper, and allow to stand in the dark for thirty minutes, unless otherwise directed in the monograph, shaking frequently. Add 10 ml of *potassium iodide solution* and 100 ml of *water*, and titrate with *0.1N sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, omitting the substance being examined, and note the number of ml of *0.1N sodium thiosulphate* required (b). Calculate the iodine value from the formula given under Method A.

The approximate weight of substance to be taken, unless otherwise directed in the monograph is calculated as follows: presumed iodine value less than 20, 1.0 g; presumed iodine value 20 to 60, 0.25 to 0.5 g; presumed iodine value 60 to 100, 0.15 to 0.25 g; presumed iodine value more than 100, 0.10 to 0.15 g.

Method C

Place an accurately weighed quantity of the substance being examined, in a dry iodine flask, add 10 ml of *carbon tetrachloride*, and dissolve. Add 25 ml of *pyridine bromide solution*, allow to stand for ten minutes in the dark and complete the determination described under Method A, beginning at the words "Place 15 ml. . . .".

The approximate weight, in g, of substance to be taken may be calculated by dividing 12.5 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

3.3.19 Peroxide Value

The peroxide value is the number which expresses in milli-equivalents of active oxygen, the amount of peroxide contained in 1000 g of the substance.

Weigh 5.0 ± 0.05 g into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of *glacial acetic acid* and 2 volumes of *chloroform*, swirl until dissolved, and add 0.5 ml of *saturated potassium iodide solution*. Allow to stand for exactly one minute, with occasional shaking, add 30 ml of *water* and titrate gradually, with continuous and vigorous shaking, with *0.01N sodium thiosulphate* until the yellow colour almost disappears, add 0.5 ml of *starch solution* and continue the titra-

tion, shaking vigorously until the blue colour just disappears (a ml). Carry out a blank determination under the same conditions without the substance being examined (b ml). The volume of *0.01 N sodium thiosulphate* in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression :

$$\text{Peroxide Value} = \frac{(a-b) \times 10}{w}$$

where

w = weight in g of the substance

3.3.20 Saponification Value

The saponification value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralise the free acids and to saponify the esters present in 1 g of substance.

Introduce about 2 g of the substance to be examined, accurately weighed, into a 200-ml flask of borosilicate glass fitted with a reflux condenser. Add 25 ml of *0.5 N alcoholic potassium hydroxide*, accurately measured, and boil under reflux on a water-bath for 30 minutes, frequently rotating the contents. Add 1 ml of *phenolphthalein solution* and titrate immediately with *0.5 N hydrochloric acid*. Note the volume in ml of titrant (a).

Carry out a blank test under the same conditions, omitting the substance. Note the volume in ml of titrant (b), calculate the saponification value from the formula :

$$\text{Saponification Value} = \frac{(b-a) \times 28.05}{w}$$

where

w = weight in g of the substance

NOTE—If the oil has been saturated with carbon dioxide for the purpose of preservation, expose it in a shallow dish in a vacuum desiccator for 24 hours before weighing the sample.

3.3.21 Unsaponifiable Matter

The unsaponifiable matter consists of substances present in oils and fats which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the substance to be examined, after saponification.

Method

Introduce about 5 g of the substance to be examined, accurately weighed, into a 250-ml flask fitted with a reflux condenser. Add a solution of 2 g of *potassium hydroxide* in 40 ml of *alcohol* and heat on a water-bath for one hour, shaking frequently. Transfer the contents of the flask to a separating funnel with the aid of 100-ml of hot *water* and while the liquid is still slightly warm, shake very carefully with three quantities, each of 100 ml, of *solvent ether*. Combine the ether extracts in a second separating funnel containing 40 ml of *water*, swirl gently for a few minutes, allow to separate and reject the lower layer; wash the ether extract with two quantities, each of 40 ml, of *water* and with three quantities, each 40 ml, of a 3 per cent w/v solution of *potassium hydroxide*, each treatment being followed by a washing with 40 ml of *water*. Finally, wash the ether layer with successive quantities, each of 40 ml of *water* until the aqueous layer is no longer alkaline to *phenolphthalein*. Transfer the ether layer to a weighed flask, washing out the separating funnel with *solvent ether*. Distil off the ether and add to the residue 6 ml of *acetone*. Remove the solvent completely from the flask with the aid of a gentle current of air. Dry at 100° - 105° for 30 minutes. Cool in a desiccator and weigh the residue. Calculate the unsaponifiable matter as a percentage (w/w).

Dissolve the residue in 20 ml of *alcohol*, previously neutralised to *phenolphthalein solution*, and titrate with *0.1 N alcoholic sodium hydroxide*. If the volume of *0.1 N alcoholic sodium hydroxide* exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter, and the test must be repeated.

3.3.22 Analysis of Vegetable Drugs

Sampling

Original samples : (a) Samples of crude vegetable drugs is which the component parts are 1 cm or less in any dimension and of powdered or ground drugs may be taken by means of a sampling device that removes a core from the top to the bottom of the container. Not less than two cores are taken in opposite directions.

When the total weight of the drug to be sampled is less than 100 kg, at least 250 g are withdrawn to constitute an *original sample*.

When the total weight of the drug to be sampled is more than 100 kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of the quarters weighs at least 125 g; two such quarters then constitute an *original sample*.

(b) Samples of crude vegetable drugs in which the component parts are over 1 cm in any dimension may be taken by hand.

When the total weight of the drug to be sampled is less than 100 kg, samples are taken from different parts of the container or containers. Not less than 500 g of samples so taken constitute an *original sample*.

When the total weight of the drug to be sampled is more than 100 kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of

the quarters weigh not less than 250 g; two such quarters then constitute an *original sample*.

NOTE – Where the total weight of crude drug to be sampled is less than 10 kg, the preceding methods may be followed but somewhat smaller quantities are to be withdrawn but in no case shall the original samples weigh less than 125 g.

Test sample : Withdraw as much as may be necessary of the *original sample* by quartering, taking care to see that the portion is representative of the gross sample. In the case of unground or unpowdered drugs, grind the sample so that it will pass through a No. 22 *sieve*. If the sample cannot be ground, it should be reduced to as fine a state as possible. Mix by rolling it in paper or cloth, spread it out in a thin layer, and withdraw the portion for analysis.

Foreign Organic Matter

Foreign organic matter is the material consisting of any or all of the following:

(i) parts of the organ or organs from which the drug is derived other than the parts named in the definition and description or for which a limit is prescribed in the individual monograph; (ii) any organs, other than those named in the definition and description; (iii) Moulds, insects or other animal contamination.

Method : Weigh 100 to 500 g or the quantity specified in the monograph, of the *original sample* and spread it out in a thin layer. Inspect the sample with the unaided eye or with the use of a 6 $\bar{x}$ lens and separate the foreign organic matter by hand as completely as possible. Weigh it and determine the percentage of foreign organic matter, calculated from the weight of the drug taken. Use the maximum quantity of sample for coarse or bulky drugs.

Alcohol-soluble Extractive

Macerate 5.0 g of the air-dried drug, coarsely powdered, with 100 ml of *alcohol* of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly taking precautions against loss of alcohol, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105°, and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Water-soluble Extractive

Method I : Proceed as directed for the determination of **Alcohol-soluble Extractive**, using *chloroform water* instead of *alcohol*.

Method II : Add 5.0 g to 50 ml of *water* at 80° in a stoppered flask. Shake well and allow to stand for ten minutes; cool to 15° and add 2 g of *kieselguhr*; filter. Transfer 5 ml of the filtrate to a tared evaporating basin 7.5 cm in diameter, evaporate the solvent on a water-bath, continue drying for half an hour, finally dry in a steam oven for two hours and weigh the residue. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Ash

Use Method I for crude vegetable drugs and Method II for other substances.

Method I : Weigh accurately a quantity of the *test sample* equivalent to 2 to 3 g of the air-dried drug in a tared platinum or silica dish, and incinerate at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot *water*, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°. If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15 ml of *alcohol*, break up the ash with a glass-rod, burn off the alcohol, and again heat the whole to a dull red heat. Cool, weigh the ash and calculate the percentage of ash with reference to the air dried drug.

Method II : Weigh accurately about 1 g of the substance being examined and carry out Method I. Calculate the percentage of ash.

Acid-insoluble Ash

Use Method I unless otherwise directed in the individual monograph.

Method I : Boil the ash obtained as directed under *Ash*, above, with 25 ml of *2N hydrochloric acid*, for five minutes, collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot *water*, ignite, and weigh. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Method II : Place the *ash* or the *sulphated ash*, as directed in the monograph, in a crucible, add 15 ml of *water* and 10 ml of *hydrochloric acid*, cover with a watch-glass, and boil for ten minutes; allow to cool. Collect the insoluble matter on an ashless filter paper, wash with hot *water* until the filtrate is neutral, ignite to dull redness, cool in a desiccator and weigh. Reheat until the difference between the two successive weighings is not more than 1 mg. Calculate the percentage of acid-soluble ash with reference to the air-dried drug.

Water-soluble Ash

Boil the *ash* for five minutes with 25 ml of *water*; collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot *water*, and ignite for fifteen minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Water

Weigh accurately about 10 g of the *test sample* in a tared evaporating dish and dry in an oven at 105° for five hours. Cool and weigh. Continue the drying and weighing at one hour intervals until the difference between two successive weighings corresponds to not more then 0.25 per cent.

Continuous Extraction of Drugs

Where continuous extraction of a drug or any other substance is recommended in the monograph, the process consists of percolating it with a suitable solvents at a temperature approximately that of the boiling-point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapour of the solvent around the percolator may be used. The type commonly known as the soxhlet apparatus is suitable for this purpose.

A simple apparatus is shown in the accompanying illustration. A is an outer tube of stout glass; the wider part is about 18 cm in length and has an internal diameter of 4.8 to 5 cm; the lower end C is about 5 cm in length and has an external diameter of about 1.6 cm. B is a straight glass tube open at both ends, about 9 cm in length and having an external diameter of about 3.8 cm; over its lower flanged end is tied firmly a piece of calico or other suitable material. D is a glass coil, which supports the margin of the tube B and prevents it from resting in contact with the outer tube A. The lower end C of the outer tube A is fitted by a cork to the distilling flask E, in which a suitable quantity of the solvent has been placed. The substance to be extracted, previously moistened with the solvent or subjected to any preliminary treatment required, is introduced into the inner tube B, which is supported so that the percolate drops into the outer tube. A pad of cotton wool G is placed on the top of the drug, the inner tube is lowered into position and the outer tube connected by means of a suitable cork with the tube of a reflux condenser F. The flask is heated and the extraction continued as directed (*see* Fig. 3).

Complete Extraction of Alkaloids

Complete extraction is indicated by the following tests:

When extracting with an aqueous or alcoholic liquid – After extracting at least three times with the

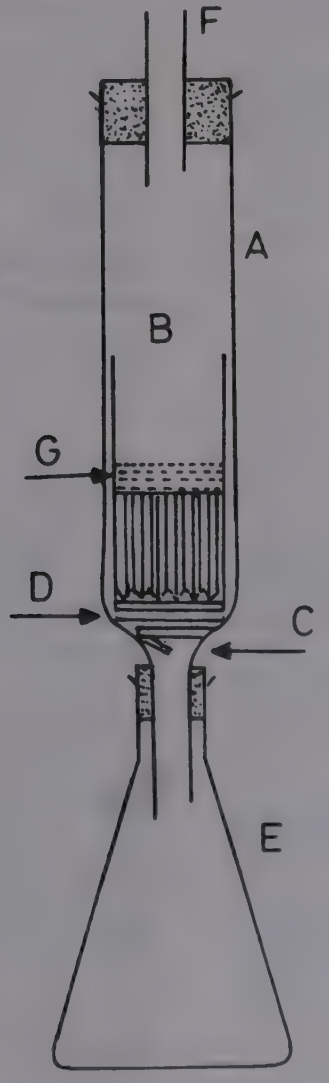


Fig. 3 APPARATUS FOR THE CONTINUOUS EXTRACTION OF DRUGS

liquid, add to a few drops of the next portion, after acidifying with *2N hydrochloric acid* if necessary, 0.05 ml of *potassium mercuri-iodide solution* or, for solanaceous aklaloids, 0.05 ml of *potassium iodobismuthate solution*; no precipitate, or turbidity, is produced.

When extracting with an immiscible solvent – After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of *0.1N hydrochloric acid*, remove the organic solvent by evaporation, transfer the aqueous residue to a test-tube, and add 0.05 ml of *potassium mercuri-iodide solution* or for solanaceous alkaloids, 0.05 ml of *potassium iodobismuthate solution* or, for emetine, 0.05 ml of *iodine solution*; not more than a very faint opalescence is produced.

3.3.23 Purity of Vegetable Oils

1. Absence of cottonseed oil in other oils

Mix in a stout glass tube, having a capacity of not less than 15 ml, 2.5 ml of the oil, 2.5 ml of *amyl alcohol*, and 2.5 ml of a 1 per cent w/v solution of *precipitated sulphur* in *carbon disulphide*. Close the tube tightly, and immerse to one-third of its depth in boiling water; no pink or red colour develops within thirty minutes.

2. Absence of sesame oil in other oils

Shake 2 ml of the oil with 1 ml of *hydrochloric acid* containing 1 per cent w/v of *sucrose*, and allow to stand for five minutes, the acid layer is not coloured pink, or if a pink colour develops, it is not deeper than that obtained by repeating the test without the sucrose.

3.3.24 Vitamin Assays

3.3.24.1 DETERMINATION OF NICOTINIC ACID OR NICOTINAMIDE

Special Reagents

Cyanogen bromide solution : Dissolve 5g of *cyanogen bromide* in sufficient *water* to produce 50 ml.

CAUTION – Prepare the solution under a hood since cyanogen bromide volatilises at room temperature and the vapour is highly poisonous.

Sulphanilic acid solution : To 2.5 g of *sulphanilic acid* add 15 ml of *water* and 3 ml of *dilute ammonia solution*. Mix, add, with stirring, more *dilute ammonia solution*, if necessary, until the acid dissolves, adjust the pH of the solution to about 4.5 with *dilute hydrochloric acid*, using *bromocresol green solution* as an external indicator and dilute with *water* to 25 ml.

Ammonia solution : Dilute 2 ml of *strong ammonia solution* to 100 ml with *water*.

Stock nicotinic acid or stock nicotinamide solution : Weigh accurately 50 mg of *nicotinic acid R.S.* or *nicotinamide R.S.*, previously dried in an oven at 105° for two hours and stored, protected from light, in a desiccator over *phosphorus pentoxide*. Dissolve in sufficient *alcohol (25 per cent)* to produce 500.0 ml. Each ml of this solution contains 100 µg of nicotinic acid or nicotinamide. Store in a refrigerator.

Standard solution : Dilute 10.0 ml of *stock nicotinic acid solution* or *stock nicotinamide solution* with sufficient *water* to produce 100.0 ml. Each ml of this solution contains 10 µg of *nicotinic acid* or *nicotinamide*.

Test solution : Prepare as directed in the individual monograph.

Method

Pipette into four marked tubes the quantities of the appropriate *standard solution*, the *test solution*, *ammonia solution*, and *water* indicated in the following Table.

Constituent	Tube 1 (ml)	Tube 2 (ml)	Tube 3 (ml)	Tube 4 (ml)
Standard solution	1.0	1.0	–	–
Test solution	–	–	1.0	1.0
Ammonia solution	0.5	0.5	0.5	0.5
Water	6.5	1.5	6.5	1.5
Cyanogen bromide solution	–	5.0	–	5.0
Sulphanilic acid solution	2.0	2.0	2.0	2.0
Hydrochloric acid	One drop	–	One drop	–

To tube 1 add the *sulphanilic acid solution*, shake well, add the *hydrochloric acid*, mix, place in a suitable spectrophotometer and adjust to zero *extinction* at 450 nm, Appendix 5.15 A. To tube 2 add the *cyanogen bromide solution*, mix and exactly 30 seconds after completion of the addition of the *cyanogen bromide solution* add the *sulphanilic acid solution*, with swirling. Close the tube and after two minutes measure the *extinction* of a 1-cm layer of the resulting solution against solution from tube 1 as the blank. Repeat the procedure with tubes 3 and 4. Calculate the quantity of nicotinic acid or nicotinamide in 1 ml of the *test solution* and from this figure calculate the quantity of nicotinic acid or nicotinamide in the sample.

3.3.24.2 DETERMINATION OF RIBOFLAVINE

NOTE – Protect the solutions from direct sunlight at all stages.

Special Reagents

Riboflavine stock solution : Weigh accurately 50 mg of *riboflavine R.S.* previously dried at 105° for two hours and stored protected from light in a desiccator over *phosphorus pentoxide*. Add about 300 ml of *0.02N acetic acid* and heat on a water bath with frequent agitation until the riboflavine has dissolved. Cool and add sufficient *0.02N acetic acid* to produce 500.0 ml. Store under *toluene* in a refrigerator. Dilute an accurately measured volume of this solution with *0.02N acetic acid* to produce a solution containing 10.0 µg riboflavine per ml to obtain *riboflavine stock solution*. Store under *toluene* in a refrigerator.

Standard solution : Dilute 10.0 ml of *riboflavine stock solution* with sufficient *water* to produce 100.0 ml. Each ml of standard solution contains 1.0 µg of riboflavine.

Test solution : Weigh accurately a suitable quantity of the substance being examined equivalent to about 100 µg of riboflavine, add 25 ml of *0.1N hydrochloric acid* and shake vigorously for a few minutes. Wash down the sides of the flask with *0.1N hydrochloric acid* and heat in an autoclave at 121° to 123° for thirty minutes. Cool and adjust the pH to 6.0 to 6.5, with vigorous agitation, with *N sodium hydroxide*, then add *0.1N hydrochloric acid* immediately until no further precipitation occurs (pH, approximately 4.5). Dilute the mixture with sufficient *water* to produce a solution containing not less than 0.1 µg of riboflavine per ml. Filter through paper which will not adsorb riboflavine and discard the first few ml of the filtrate. To a suitable volume of the filtrate add, with vigorous shaking, *0.1N sodium hydroxide* to produce a pH of 6.6 to 6.8 and dilute with *water* to produce a solution containing approximately 0.1 µg of riboflavine per ml.

Method

To each of four or more tubes add 10.0 ml of the *test solution*. To each of two or more of these tubes add 1.0 ml of *standard solution*, mix, and to each of two or more of the remaining tubes add 1.0 ml of *water*, and mix. To each tube add 1.0 ml of *glacial acetic acid*, mix, then add, with mixing, 0.5 ml of a 4 per cent w/v solution of *potassium permanganate* and allow to stand for two minutes. To each tube add, with mixing, 0.5 ml of *hydrogen peroxide solution*. Shake the tubes vigorously until excess oxygen is expelled. Remove any gas bubbles remaining on the sides of the tubes after foaming has ceased, by tipping the tubes so that the solution flows slowly from end to end. Measure the *fluorescence* of all the tubes in a suitable fluorimeter fitted with an input filter of narrow transmittance range with a maximum at about 440 nm and an output filter of narrow transmittance range with a maximum at about 565 nm, Appendix 5.15 C. Note the average reading from the tubes containing the *test solution* as T_u and the average reading from the tubes containing both the *test solution* and the *standard solution* as T_s. To each of one or more tubes of each kind add, with mixing, 20 mg of *sodium hydrosulphite*, and within five seconds again measure the *fluorescence*, disignating the average reading as T_b.

Calculate the quantity in mg of riboflavine in each ml of the *test solution* from the expression

$$\frac{0.0001(T_u - T_b)}{(T_s - T_u)}$$

Calculate the quantity of riboflavine in the sample.

3.3.24.3 DETERMINATION OF THIAMINE

Special Reagents

Potassium chloride solution : Dissolve 25 g of *potassium chloride* in sufficient *0.1N hydrochloric acid* to produce 100 ml.

Alkaline potassium ferricyanide solution : Dilute 4 ml of *potassium ferricyanide solution* to 100 ml with a 15 per cent w/v solution of *sodium hydroxide* in *water*.

NOTE – This solution must be used within four hours of preparation.

Thiamine solution : Weigh accurately 50 mg of *thiamine hydrochloride R.S.* and dissolve in sufficient *0.005N hydrochloric acid* to produce 250.0 ml. Each ml of this solution contains 0.2 mg of thiamine hydrochloride.

Method

(a) Preparation of calibration curve : Dilute 5.0 ml of *thiamine solution* to 100.0 ml with *0.1N hydrochloric acid* and dilute 10.0 ml of the solution to 100.0 ml with *potassium chloride solution*. Dilute 1.0 ml, 2.0 ml and 4.0 ml of this solution with sufficient *potassium chloride solution* to produce three solutions, each of 5 ml; to each solution contained in a small grease-free separator, add 3 ml of *alkaline potassium ferrycyanide solution*, shake for one minute, add 25 ml of *isobutyl alcohol* saturated with *water*, shake vigorously for ninety seconds, and allow to separate; to the alcoholic layer add 1 ml of *ethyl alcohol* and measure the *fluorescence* in a suitable fluorimeter fitted with filters having maximum transmittance between 400 nm and 450 nm, Appendix 5.15C, using a 0.0001 per cent w/v solution of *quinine sulphate* in *0.1N sulphuric acid* for comparison, if necessary.

Prepare a calibration curve from the figures obtained.

(b) Estimation of thiamine : Weigh accurately about 2 g, add 20 ml of *0.2N hydrochloric acid* and mix thoroughly. Wash down the sides of the container with a small quantity of *0.2N hydrochloric acid*, and heat in an autoclave at 121° to 123° for thirty minutes. Cool, and

disperse any aggregates of solid. Transfer to a 100-ml volumetric flask and dilute to volume with *water*. Transfer an aliquot of this solution, containing approximately 20 µg of thiamine hydrochloride to a 100-ml centrifuge tube. Adjust to pH 4.0 to 4.5 with *2M sodium acetate* using *bromocresol green solution* as an external indicator. Add 5 ml of a 10 per cent solution or suspension of a suitable diastatic enzyme such as alpha-amylase, and heat at 45° to 50° for three hours. Cool, clarify the mixture by centrifuging; quantitatively transfer the supernatant liquid to a 50-ml volumetric flask. Wash the residue with a 10 ml and then a 5 ml quantity of *potassium chloride solution*, centrifuging each time and adding the washing to the main extract. Dilute to volume with *potassium chloride solution* and mix. Pipette 5 ml into a small grease-free separator and continue by the method used for the **Preparation of calibration curve**, beginning at the words "add 3 ml of *alkaline ferricyanide solution*. . .". Obtain the amount of thiamine hydrochloride present by reference to the calibration curve.

3.3.24.4 ASSAY OF VITAMIN A

The following methods for the determination of the potency of Vitamin A in pharmacopoeial preparations are based on the measurement of the ultra-violet spectrum of the vitamin at specified wavelength. The potency of Vitamin A is expressed in terms of the Unit, which is 0.344 µg of all-trans-Vitamin A acetate, equivalent to 0.3 µg of all-trans-Vitamin A alcohol.

A simple measurement of the absorption maximum is not sufficient because in addition to Vitamin A, other substances may contribute to the measured extinction. The irrelevant absorption of such substances may be taken care of under certain conditions (given below) by the use of correcting equations. In other cases, where these conditions are not satisfied, preliminary treatment by chemical or physical methods or by a combination of these methods may be necessary before the following spectrophotometric methods are used to give a valid assay.

The assay should be carried out as speedily as possible and care must be taken to avoid exposure to actinic light and to oxidising agents.

Since the position of the absorption maximum is an important criterion and since the correcting equations require measurements at exact wavelengths, it is essential that the wavelength scale of the spectrophotometer should be checked immediately before the assay. The mercury lines at 313.16 and 334.15 nm provide suitable points and for convenience the setting of the instrument on these lines may be related to its setting on the hydrogen lines at 379.7 and 496.1 nm. The precision of a corrected extinction is appreciably less than that of the three directly determined *extinction*, from Appendix 5.15 A, which it is calculated and the extinction measurements therefore require special care and not fewer than two assays should be performed.

Method A

This method is suitable for preparations wich contain vitamin A as an ester and in a form directly soluble in *cyclohexane*.

Special Reagent

Cyclohexane : The *extinction* of a 1-cm layer in a silica cell at or above 328 nm when measured against *water* does not exceed 0.1, Appendix 5.15 A.

Method : Dissolve an accurately weighed quantity of the sample, or a prepared fraction thereof, in sufficient the *cyclohexane* to give a solution containing between 9 and 15 Units of Vitamin A per ml. Measure the *extinction*, Appendix 5.15 A, of the solution against the *cyclohexane* at the wavelengths given in the Table 1. Ascertain the wavelength where maximum absorption occurs.

TABLE 1

Wavelength (nm)	Relative Extinction
300	0.555
316	0.907
328	1.000
340	0.811
360	0.299

Calculate the extinctions at the wavelengths specified in the Table 1 as fractions relative to that at 328 nm. Calculate also the extinction at 328 nm in terms of E (1 per cent, 1 cm) for the sample.

If the wavelength of maximum absorption lies between 326 and 329 nm and the relative extinctions are within 0.02 of those in the Table 1, calculate the Vitamin A potency of the sample in Units per g from the expression:

$$E(1\text{ per cent, }1\text{-cm})\text{ at }328\text{ nm} \times 1900$$

If the wavelength of maximum absorption lies between 326 and 329 nm but the relative extinctions are not within 0.02 of those in the Table, calculate a corrected extinction at 328 nm using the following expression:

$$E_{328}(\text{corrected}) = 3.52(2E_{328} - E_{316} - E_{340})$$

$$\text{Calculate the ratio } \frac{E_{328}\text{ (corrected)}}{E_{328}\text{ (gross)}}$$

If the ratio lies between 0.97 and 1.03, the E_{328} (corrected) may be ignored. Calculate the Vitamin A potency of the sample in Units per g from the expression:

$$E\text{ (1 per cent, 1-cm) at }328\text{ nm} \times 1900$$

If the ratio lies betwenn 0.85 and 0.97, calculate the Vitamin A potency of the sample in Units per g from the expression:

E (1 per cent, 1-cm) corrected at 328 nm × 1900

If the ratio lies outside the range 0.85 to 1.03 or the wavelength of maximum absorption does not lie between 326 and 329 nm, the sample must be treated as described under Method B.

Method B

Special Reagents

(1) Ether, anaesthetic : Freshly redistilled, discarding the first and last 10 per cent portions.

(2) Isopropyl alcohol : The *extinction* of a 1-cm layer in a silica cell when measured against *water* is not greater than 0.01 in the range 320-350 nm and is not greater than 0.05 at 300 nm, Appendix 5.15 A.

Method : Weigh accurately a quantity of the sample containing not less than 500 Units of Vitamin A and not more than 1 g of fat. Add about 50 mg of *hydroquinone*, 30 ml of *ethyl alcohol*, and 3 ml of a 50 per cent w/w solution of *potassium hydroxide*. Boil gently under a reflux condenser in a stream of *oxygen-free nitrogen* for thirty minutes, cool rapidly, and add 30 ml of *water*. Transfer to a separator with the aid of three quantities, each of 50 ml, of *anaesthetic ether*, add 2 g of finely-powdered *sodium sulphate*, and extract the vitamin A by shaking for 2 minutes. After complete separation, discard the aqueous layer and wash by swirling gently with 50 ml of *water*. Allow the two layers to separate completely and discard the water. Add a further 50 ml of *water* and gently tumble the separator for 2 minutes. Allow the two layers to separate completely and again discard lower aqueous layer. Repeat the washing with two further 50 ml portions of *water*, shaking vigorously. Discard the aqueous layers and filter the ether solution through a small plug of cotton wool, previously washed with *anaesthetic ether*, into a 200-ml volumetric flask. Wash the separator and filter with *anaesthetic ether* and add the washings to the ether in the flask. Dilute the combined ether extracts to volume with *anaesthetic ether* and mix well.

Evaporate a 25-ml portion of the ether extract to about 5 ml without applying heat and with the aid of a stream of *oxygen-free nitrogen* or vaccum. Continue the evaporation to about 3 ml. Dissolve the residue in sufficient *isopropyl alcohol* to produce a solution containing between 9 and 15 Units of vitamin A per ml. Measure the *extinctions* at 300, 310 and 334 nm Appendix 5.15 A. Ascertain the wavelength where maximum absorption occurs.

If the wavelength of maximum absorption lies between 323 and 327 nm and the extinction at 300 nm relative to that at 325 nm does not exceed 0.73, a corrected extinction at 325 nm is derived from the expression:

$$E_{325}(\text{corrected}) = 6.815\ E_{325} - 2.555\ E_{310} - 4.260\ E_{334}$$

$$\text{Calculate the ratio } \frac{E\ (\text{corrected})}{E_{325}\ (\text{gross})}$$

If the ratio lies between 0.85 and 0.97 calculate the Vitamin A potency of the sample in Units per g from the expression:

E (1 per cent , 1-cm) at 325nm × 1830

If the wavelength of maximum absorption lies outside the range 323-327 nm or if the ratio lies outside the range 0.85–1.03 or if the extinction at 300 nm relative to that at 325 nm exceeds 0.73, the unsaponifiable fraction of the sample must be chromatographed.

All-trans-Vitamin A Acetate

Description : White to very pale yellow, free-flowing crystals.

Solubility : Insoluble in *water*; completely soluble in *alcohol*, in *chloroform* in *solvent ether*, in *light petroleum*, in fats, and in fixed oils.

Light absorption : *Extinction* of a 1-cm layer of a 0.0003 per cent w/v solution in *isopropyl alcohol* at 325 nm not less than 0.4575, in *cyclohexane* at 327.5 nm, not less than 0.4545, and in *ethyl alcohol* at 326 nm, not less than 0.4635, Appendix 5.15 A.

Melting range : 57.0° to 60.0°, Appendix 5.11.

Relative extinctions : Measure the *extinction*, Appendix 5.15 A, of a 1-cm layer of 0.0003 per cent w/v solution in *cyclohexane* at 327.5 nm and at the following wave lengths; the relative *extinctions*, calculated with reference to the *extinction* at 327.5 nm, are within the limits stated:

Wavelength (nm)	Relative Extinction
300	0.545–0.565
312.5	0.845–0.865
337.5	0.845–0.865
345	0.685–0.705
360	0.290–0.310

3.3.24.5 ASSAY OF VITAMIN D

The following method is suitable for the determination of Vitamin D as an ingredient of pharmacopoeial preparations:

The assay should be completed promptly and care

should be taken throughout the procedure to keep to a minimum the exposure to air and to actinic light by the use of inert gas and low actinic glassware. All the operations should be carried out in subdued light.

Special Reagents

(1) Adsorbent : A chromatographic grade of Kaolin such as Florex XXS or of fuller's earth having a water content corresponding to not less than 8.5 per cent and not more than 9.0 per cent of loss on drying at 105° for 6 hours.

NOTE – Adjust the water content, if necessary, by drying in vacuum at room temperature, restoring the water required and equilibrating by shaking for 2 hours.

(2) Ethylene chloride : Purify by passing through a column of granular silica gel.

(3) Standard preparation of Vitamin D : Dissolve 0.01 g of *ergocalciferol R.S.* or *cholecalciferol R.S* (for assaying substances labelled to contain Vitamin D as ergocalciferol, or as cholecalciferol, respectively) in sufficient *ethylene chloride* to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *ethylene chloride* to give a solution containing 10 micrograms (400 Units) of Vitamin D per ml.

Chromatographic Tubes

Column No.1 : A chromatographic tube 25 cm long, 2.5 cm (internal) in diameter and to which is fitted at the lower end, a sintered glass disc or a small plug of glass-wool and a tap.

Column No. 2 : A chromatographic tube 20 cm long, 5 mm in diameter and to which is fitted at the lower end, a sintered glass disc and a tap.

Chromatographic Columns

Column No. 1 : Shake 200 ml of *trimethylpentane* with sufficient *polyethylene glycol 600* so that, on separation, two layers are obtained, to 100 ml of the supernatant liquid add 25 g of *chromatographic siliceous earth*, shake vigorously, to form a thin slurry, add, in small portions and with vigorous stirring, 10 ml of *polyethylene glycol 600* and continue to stir for a further two minutes to produce a uniform suspension.

Transfer the suspension in small portions to the chromatograph tube, apply gentle suction and pack each portion carefully with the aid of a glass plunger; add sufficient of the suspension to produce a column 15 cm in length and discard any eluate.

Standardisation of column – Determine the volume in which ergocalciferol is recovered from the column by the following method:

Transfer 2 ml of a 0.03 per cent w/v solution of *ergocalciferol* in *trimethylpentane* to the top of the column and rinse it into the column with not more than 5 ml of *trimethylpentane*; elute the column with *trimethylpentane*, adjusting the rate of flow from the bottom of the column to 2 to 3 ml per minute and collect successive 5 ml fractions of the eluate.

Measure the extinction of a 1-cm layer of each fraction at the maximum at about 263 nm and hence determine the position, relative to volume of eluate, at which the elution of calciferol begins and finishes.

Column No. 2 : Mix 5 g of adsorbent with sufficient *trimethylpentane* to form a slurry and transfer the slurry to the chromatograph tube. Pack the tube carefully with the aid of a glass plunger and discard the eluate.

Sample Preparation

Accurately weigh a quantity of the sample to be assayed, equivalent to about 400 Units of Vitamin D. For capsules, the mixed contents of 20 capsules may be used as the sample. Add 10 ml of a freshly prepared 0.01 per cent w/v solution of *butylated hydroxytoluene* in *alcohol*, 15 ml of a 50 per cent w/v solution of *potassium hydroxide* in *water*, 5 ml of *alcohol* and reflux on a water-bath for 30 minutes; cool, transfer the solution to a separator with the aid of 50 ml of *water*, add 75 ml of *solvent ether*, shake vigorously, allow to separate, transfer the aqueous layer to a second separator and extract with three successive quantities, each of 30 ml, of *solvent ether*, adding each ethereal extract to the liquid in the first separator and finally discarding the aqueous solution.

Pour two successive quantities, each of 100 ml, of *water* through the ethereal solution without shaking and discard the aqueous layers; add successive 10-ml quantities of water to the ethereal solution, agitating gently each time, and discarding the aqueous extracts, continuing the process until the aqueous extracts are neutral to phenolphthalein solution.

Dry the ethereal solution by stirring with *anhydrous sodium sulphate*, decant the ethereal solution, wash the residue with successive small portions of *solvent ether*, and evaporate the combined solution and washings on a water-bath to a volume of about 5 ml; cool and evaporate to dryness in a current of *nitrogen*. Dissolve the residue in 5.0 ml of *trimethylpentane* to obtain the sample preparation.

Procedure

Transfer the sample preparation to the top of chromatographic column No. 1 with the aid of 5 ml of *trimethylpentane*; elute the column with *trimethylpentane*, adjusting the rate of flow of eluate from the bottom of the column to 2 to 3 ml per minute, and collect the fraction of eluate estimated to contain the calciferol, as indicated by the standardisation of the column.

Transfer the eluate collected from chromatographic column No. 1 to the top of chromatographic column No. 2, allow the liquid to flow, add to the top of the column 10 ml of *trimethylpentane* and discard the eluate. Elute the column with 50 ml of *benzene*, evaporate the eluate on a water-bath to a volume of about 5 ml, cool, evaporate to dryness in a current of *nitrogen* and dissolve the residue in 4.0 ml of *ethylene chloride* to obtain the sample solution.

Add 1.0 ml of the sample solution to each of three tubes; to the first tube add 1.0 ml of a mixture of equal volumes of *ethylene chloride* and *acetic anhydride* and 10.0 ml of *antimony trichloride solution* in *ethylene chloride*, to the second tube add 1.0 ml of *ethylene chloride* and 10.0 ml of *antimony trichloride solution* in *ethylene chloride* and to the third tube add 1.0 ml of *standard preparation of Vitamin D* and 10.0 ml of *antimony trichloride solution* in *ethylene chloride*.

Measure the *extinction* of a 1-cm layer of each solution, exactly 1 minute after the addition of the antimony trichloride solution at the maximum at about 500 nm, using *ethylene chloride* as the blank, Appendix 5.15 A.

The amount of Vitamine D, in mg, in the weight of sample taken is given by the formula:

$$\frac{0.04(e_2-e_1)}{(e_3-e_2)}$$

where

e_1 = the *extinction* due to the solution in the first tube,

e_2 = the *extinction* due to the solution in the second tube,

e_3 = the *extinction* due to the solution in the third tube.

Each mg of ergocalciferol or cholecalciferol is equivalent to 40,000 Units of Vitamin D.

3.3.25 Determination of Water

The following titrimetric method is suitable for determining the water content of many pharmacopoeial substance in which water is present either as water of hydration or in absorbed form.

The method is based on the stoichiometric reaction between water and a solution of sulphur dioxide and iodine in pyridine and methyl alcohol (Karl Fischer reagent). The reagent and the solutions used in this method must be kept anhydrous and precautions must be taken throughout to prevent exposure to atmospheric moisture.

In colourless solutions, the end-point of the titration may be determined electrometrically or visually by a change from a canary yellow to an amber colour. In coloured solutions the following electrometric methods must be used.

Apparatus

A titration vessel, of about 60-ml capacity, is fitted with two platinum electrodes, about 0.05 sq. cm in area and about 2.5 cm apart, a nitrogen inlet tube, a stopper which accommodates the burette tip and a vent tube protected by a suitable desiccant such as *phosphorous pentoxide* or *silica gel*. The substance to be examined is introduced through an inlet or side arm which can be closed by a ground stopper. Stirring is done magnetically or by means of a stream of dried *nitrogen* passed through the solution during the titration. The air in the entire system should be kept dry during the titration.

The voltage across the electrodes may be obtained from a 1.5-volt dry cell and a variable resistance of about 2000 ohms. The resistance is adjusted so that an initial current passes through the electrodes connected in series to an ammeter. On adding the reagent the needle of the ammeter shows a deflection but returns immediately to its starting position. At the end-point of the titration a slight excess of the reagent produces a deflection which persists for not less than half a minute.

Karl Fischer Reagent

Mix in a 750-ml combustion flask 400 ml of *dehydrated methyl alcohol* and 80 g of *dehydrated pyridine*. Immerse the flask in ice and bubble dried *sulphur dioxide* slowly through the mixture until its weight has increased by 20 g. Add 45 g of *iodine* and shake until it dissolves. Allow the solution to stand for 24 hours before use. This solution deteriorates gradually; therefore standardise it within one hour before use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light and under refrigeration. A commercially available, stabilised solution of Karl Fischer reagent may be used.

Primary standardisation of the reagent : Place about 36 ml of *methyl alcohol* in the titration vessel, and add sufficient *Karl Fischer reagent* to give the characteristic end-point. Add quickly 150 to 350 mg of *sodium tartrate* ($C_4H_4N_2O_6,2H_2O$) accurately weighed, by difference, and titrate to the end-point. The water equivalence factor F, in mg of water per ml of reagent, is given by the formula 0.1566 w/v, in which w is the weight in mg, of the sodium tartrate, and v is the volume in ml, of the reagent required.

Secondary standardisation of the reagent : The reagent may alternatively be standardised for each day's use against a water-methyl alcohol solution standardised as follows: Add 2.0 ml of *water* to 1000.0 ml of *methyl alcohol*. Retain a portion of the methyl alcohol for a blank determination. Place 25 ml, accurately measured, of the water-methyl alcohol solution in the titration vessel and titrate with *Karl Fischer reagent*. Similarly, carry out a blank titration on 25 ml, accurately measured of the methyl alcohol used, and make any necessary correction.

The water content in mg per ml, of the water-methyl alcohol solution is given by the formula VF/25, in which V is the volume in ml of *Karl Fischer reagent* required and F is the water equivalence factor of the reagent determined against *sodium tartrate* as directed under Primary Standardisation of the Reagent.

Procedure

Unless otherwise prescribed, carry out the determination of water by Method A.

Method A : Add about 20 ml of *dehydrated methyl alcohol* to the titration vessel and titrate to the electrometric end-point with the *Karl Fischer reagent*. Transfer quickly the amount of the substance to be examined, accurately weighed, to the titration vessel. Stir for one minute, and titrate again to the electrometric end-point using the *Karl Fischer reagent*.

The water content of the sample in mg, is given by S & F, in which S is the volume in ml, of reagent used to titrate the sample and F is the water equivalence factor.

Method B : The method should be followed for samples that react with difficulty or too slowly for convenient direct titration with the reagent.

Add about 10 ml of *dehydrated methyl alcohol* to the titration vessel and titrate to the electrometric end-point with the *Karl Fischer reagent*. Transfer quickly the prescribed amount, accurately weighed, of the substance to be examined followed by an accurately measured amount of *Karl Fischer reagent*, sufficient to give an excess of about 1 ml. Allow to stand, protected from light, for one minute, stirring well. Titrate the excess of reagent to the electrometric end-point with *dehydrated methyl alcohol* to which has been added an accurately known amount of *water*, equivalent to about 0.25 per cent w/v.

3.3.26 Determination of Zinc

NOTE – All reagents used in this test should have as low a content of heavy metals as is practicable. All glassware should be rinsed with warm dilute nitric acid followed by water, previously distilled in hard or borosilicate glass apparatus. Separators should not be greased with materials that dissolve in chloroform.

Special Reagents and Solvents

(1) Alkaline ammonium citrate solution : Dissolve 46 g of *citric acid* in about 70 ml of *water* and gradually add 127.5 ml of *strong ammonia solution*. Remove any heavy metals that may be present by extracting with 20-ml quantities of *dithizone extraction solution* (*see* Appendix 3.2.6) until the dithizone solution retains a clear green colour, then extract any dithizone remaining in the citrate solution by shaking with *chloroform*.

(2) Chloroform : Distil *chloroform* in hard or borosilicate glass apparatus and collect the distillate in sufficient *ethyl alcohol* to make the final concentration of alcohol 1 per cent v/v.

(3) Standard dithizone solution : Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of distilled *chloroform*. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light, and store in a refrigerator.

(4) Standard zinc solution : Weigh accurately 0.625 g of *zinc oxide*, previously ignited to constant weight, in 10 ml of *nitric acid*, and add sufficient *water* to produce 500.0 ml. Each ml of this solution contains 1.0 mg of zinc per ml.

(5) Dilute standard zinc solution : Dilute 1.0 ml of *standard zinc solution* with two drops of *nitric acid* and sufficient *water* to produce 100.0 ml. Use this solution within two weeks of preparation. Each ml of this solution contains 10µg of zinc per ml.

(6) Trichloroacetic acid solution : Dissolve 10 g of *trichloroacetic acid* in sufficient *water* to produce 100 ml.

Method

Pipette 1 to 5 ml of the preparation being examined into a centrifuge tube graduated at 40 ml. If necessary, add *0.25 N hydrochloric acid* dropwise, to obtain a clear solution. Add 5 ml of *trichloroacetic acid solution* and sufficient *water* to produce 40.0 ml. Mix well and centrifuge.

Pipette into a hard-glass separator a volume of the supernatant liquid equivalent to about 5 to 20 µg of zinc, and add *water* to produce 20 ml. Add 1.5 ml of *alkaline ammonium citrate solution* and 35 ml of *standard dithizone solution*. Shake vigorously several times and allow the chloroform to separate. Collect the chloroform extract through a cotton plug in the stem of the separator and discard the first few ml. Measure the *extinction* of a 1-cm layer of the extract at 530 nm, Appendix 5.15 A, using as the blank, an extract obtained by repeating the determination omitting the preparation being examined.

Calculate the amount of zinc by reference to a standard graph obtained by using 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of *dilute standard zinc solution* and repeating the determination as outlined above.

3.3.27 Closures for Injections

A closure for injections is a packaging component which is in direct contact with the drug. It may be of synthetic or natural origin and is generally a complex mixture of many ingredients such as the basic polymer, fillers, vulcanising agents, accelerators, stabilisers and pigments. The properties of a closure are dependent upon the mix of these ingredients and also on the method of processing them. It is therefore necessary to choose the right composition of a closure for an injectable preparation; the suitability of a

closure for its intended use can be determined only after evaluating several factors by means of additional specific tests.

Rubber closures are available in a number of formulations and consequently different closures possess different properties. It is impracticable to devise a set of standards which, if complied with, will ensure the compatibility of the closure with the injectable preparation with which it is used. A test for compatibility has, therefore, to be carried out before a rubber mix is approved. Any proposed change in the composition of an approved rubber mix should be notified to the user since further compatibility tests may become necessary.

The following test procedures apply to rubber closures which comprise wade (flat rubber discs), plugs (with or without a skirt or flange) and caps (rubber covers held in position on the outside of containers by the tension of the rubber) so as to form with their appropriate seals an effective barrier against micro-organisms after sterilisation.

Preparation of Samples

Wash the closures by agitation in a 0.2 per cent solution of an anionic surface-active agent for five minutes at room temperature. Rinse five times with *water*. Put the washed closures in a suitable container of glass or inert material and autoclave at 121 ± 1° for thirty minutes with at least five times their volume of *water*. Dry the autoclaved plugs "in vacuo at 65 ± 1°C" for twenty-four hours.

Sterilisation test : The closures 'prepared' in the foregoing manner shall not soften or become tacky and there shall be no visual change in the closure.

Fragmentation test : Half fill 20 vials with *water* free from particles and seal with the 'prepared' closures in the appropriate manner.

Using a 21 S.W.G. (0.813 mm) regular point hypodermic needle (38 mm long), pierce one closure five times normal to the surface of the closure at a speed of 20 ± 1 cm/minute, ensuring that all five piercings are within a circle 5 mm in diameter and as equidistant from each other as possible. When the fifth piercing has been made, and without withdrawing the needle from the closure, connect to the needle a small clean hypodermic syringe containing about 1 ml of *water* free from particles and inject the water into the vial. Then remove the needle and examine it carefully for any sign of blunting. If blunting is evident, discard the vial and its contents and substitute a fresh one. Repeat the above test on the remaining 19 sealed vials, using a new needle for each closure. Filter the water from each vial together with suitable washings through a Buchner filter using filter paper dyed to a contrasting colour (e.g. with methylene blue in the case of white closure). Count the number of fragments without any artificial aid. The closures comply with the test if any average of not more than two fragments of rubber per closure are found except in the case of butyl rubber closures where an average of not more than four fragments of rubber per closure are found.

Self-sealability

Method A : This test is not applicable to closures fabricated from synthetic materials.

'Prepare' the closures as described above. Half fill with *water* five vials for which the closures are intended, apply the 'prepared' closures, and seal in the appropriate manner. Take each vial in turn, invert and inject through the closure by means of a 21 S.W.G. (0.8 mm) regular point needle (38 mm long) fitted to a hypodermic syringe a volume of air equal to the volume of air in the vial. Withdraw the needle rapidly and note whether any leakage occurs. There shall be no spray of water from any of the closures and not more than small droplet remaining on the surface.

Method B : 'Prepare' the closures as described above. Half fill five vials with a 0.1 per cent w/v solution of *methylene blue* prior to closure. Make 25 successive punctures using a 21 S.W.G. (0.8 mm) regular point hypodermic needle (38 mm long) in each closure as evenly as possible within a circle 5 mm in diameter. Use a new needle for each closure. Invert each vial immediately in a separate bottle containing sufficient *water* to immerse the seal. Apply to each bottle a vacuum of 20 torr for thirty minutes and examine the seal and the water for signs of leakage through the closure. The closures shall show no signs of leakage.

The following additional tests are recommended to enable pharmaceutical manufacturers to ensure that a closure is suitable for the purpose intended. Specifications should be agreed with the manufacturer of the closures and should be revised if the closure composition or ingredient quality is altered or if the process of closure manufacture is changed.

Extractable Matter

'Prepare' a number of closures as described above. Place an appropriate number of 'prepared closures' to weigh about 20 g in a flask, add 200 ml of *water* to the flask and cover the flask with a beaker or with aluminium foil which has previously been washed with *acetone*. Add 200 ml of *water* to another flask, cover the flask with a beaker or with aluminium foil as in the case of the first flask. This flask serves as a "control blank". Autoclave both the flasks for thirty minutes at 120° to 125°. Cool the flasks and allow to stand at room temperature for three hours. Preserve the extracts for the following tests:

(a) Appearance of extract : No colour or precipitate is formed and not more than faint turbidity is noticed.

(b) Reducing substances : Pipette 50-ml aliquots of the extracts of the samples and the control blank solution into two flasks, add 2 ml of *starch solution* and titrate with

0.01N iodine to the first permanent blue colour. The difference between the two titrations is a measure of the reducing substances in the aqueous extract of the closures; it should not exceed 0.5 ml in the case of latex (unfilled) closures, 1.0 ml in the case of natural rubber (filled) blender or unblended with synthetic rubber, and 1.5 ml in the case of butyl rubber.

(c) Light transmission : Measure the *extinction* of a 1-cm layer of the extract of the sample at 620 nm, Appendix 5.15 A, against the control blank solution. The values should not deviate from the agreed standards by more than 10 per cent.

(d) Heavy metals : 20 ml of the aqueous extract of the closures comply with the *limit test for heavy metals, Method A*, Appendix 3.2.4.

pH of Aqueous Extract

Cut ten closures into 2 mm pieces and autoclave with 200 ml of *water* at 121 ± 1° for ten minutes. Discard the extract and repeat the process with 500 ml of *water* for thirty minutes. Decant the extract, cool and determine the pH of the extract, Appendix 5.10. The values should not deviate from the agreed standards by more than 0.5.

Total ash : Cut the closures into small pieces and weigh accurately about 1 g of the sample in a silica crucible Ignite at 650° ± 25° to constant weight.

Biological Test

The following test is designed to evaluate the biological response in test animals, of an extract of closures. The contact of extracting medium with the total surface of the closures, the time and temperature during extraction and the aseptic handling and storage of the extract are important. Particular care must be exercised in the preparation of the extract to be injected to prevent contamination with micro-organisms and other foreign matter.

Apparatus : Use containers such as screw-capped culture bottles or test-tubes. Where the bottles or test-tubes are closed with a screw cap, the liner of the cap should be of a suitable elastomer and the exposed surface of the liner should be completely protected with an inert solid disc 50 to 75 μm in thickness and fabricated from a material such as teflow or any other polytetrafluoroethylene resin.

All glasswares should be thoroughly rinsed with *chromic acid mixture*, Appendix 6.1, followed by prolonged rinsing with sterile *water for injection*. Containers and devices used for extraction, transfer or administration of test material should be sterile and dried by a suitable process.

Procedure : Place a selected number of intact closures in an extraction container, add 50 ml of sterile *water for injection*, cap and agitate for two or three minutes. Decant, using a stainless steel screen to hold the closures in the container. Repeat this step. Remove the caps, place the containers with the sample and the caps in an oven at a temperature of about 50° and allow to dry for not more than sixteen hours.

Place two properly prepared samples to be tested in separate extraction containers and add to each container 1 ml per 1.25 ± 10.1 cm^2 of sterile *normal saline solution* and extract by heating in an autoclave at 121° ± 1° for sixty minutes. Allow adequate time for the liquid within the container to reach the extraction temperature. Cool the container to room temperature, agitate vigorously for several minutes and then aseptically transfer the extract immediately to a dry, sterile container. Carry out the following test for systemic toxicity within the following twenty-four hours. Carry out a blank omitting the closures.

Inject intravenously 1.0 ml each of the sample extract and the blank solution into each of five healthy, not previously used albino mice weighing between 17 g and 22 g. Observe the animals immediately after injection, again four hours after injection, and then at least at 24, 48 and 72 hours. If during this period none of the animals treated with the sample extract shows a significantly greater reaction than the animals treated with the blank, the sample passes the test. If any animals treated with the sample extract show only slight signs of toxicity, and not more than one animal shows gross symptoms of toxicity or death, repeat the test using ten mice. On the repeat test, all the ten animals treated with the sample extract show no significant reaction above the animals treated with the blank.

3.3.28 Containers

Containers for pharmaceutical preparations must be chosen with care and after taking into consideration the nature of the preparations and the likely effects of transportation and storage, even for short periods, of time. Pharmaceutical containers are principally made of glass and plastic.

The general requirements for containers of various types are given in the General Notices. This section deals with the specific requirements of containers and provides standards for the materials used for making containers.

A. GLASS CONTAINERS FOR INJECTABLE PREPARATIONS

There are three types of glass containers suitable for packaging injectable preparations. The types are distinguished by the resistance to water attack of new (not previously used) glass containers, the degree of attack being determined by the amount of alkali released from the glass under the influence of the attacking medium under the conditions specified. The glass used in the manufacture of glass containers should comply with the requirements for *hydrolytic resistance* given here:

Type I glass (borosilicate glass or commonly known as neutral glass) offers a high hydrolytic resistance due to the chemical composition of the glass and Type II glass (soda-lime glass suitably de-alkalized) has a high hydrolytic resistance due to an appropriate surface treatment. Both types of glass may be used for different types of injectable preparations, depending on their physico-chemical properties. Type III glass (soda-lime glass) offers only a moderate hydrolytic resistance and is usually not used for injectable preparations, except where suitable stability test data indicates that Type III is satisfactory. Containers of Type II or Type III glass should be used once only.

Containers for injectable preparations are made from uncoloured glass except that, for substances which are extremely light-sensitive, coloured glass may be used; however, the containers should be sufficiently transparent to permit visual inspection of the contents.

Hydrolytic resistance : Glass containers should comply with the following test for hydrolytic resistance. Carry out the test on the unused containers; the number of containers to be examined and the volume of test solution to be used are given in Table 1.

Rinse each container at least twice with *water* at room temperature. Just before the test, rinse each container with freshly-distilled *water*. Fill the containers to the brim with freshly-distilled *water*, empty them and calculate the average overflow volume. Fill ampoules with freshly-distilled *water* to the maximum volume compatible with sealing them. Fill bottles to 90 per cent of their calculated overflow volume and cover them with borosilicate-glass dishes previously rinsed in freshly-distilled *water*. Place the containers in an autoclave containing water in such a way that they remain clear of the water. Close the autoclave, displace the air by passing steam for ten minutes. Raise the temperature from 100° to 121° over twenty minutes and maintain the temperature of 121° for sixty minutes. Reduce the temperature to 100° over forty minutes. Remove the containers from the autoclave and cool them in a bath or running water. Within one hour of removing from the autoclave carry out the following titration. Combine the liquid from the containers being examined and measure the quantity of test solution specified in Table 1 into a conical flask. Add 0.15 ml of *methyl red solution* for each 50 ml of liquid. Titrate with *0.01N hydrochloric acid*, taking as the end-point the colour obtained by repeating the operation using the same volume of freshly-distilled *water*. The difference between the titrations represents the volume of *0.01N hydrochloric acid* required by the test solution. Calculate the volume of *0.01N hydrochloric acid* required for each 100 ml of test solution, if necessary. The result is not greater than the value given in Table 2.

Distinction of Types I and II glass : Examine the number of containers indicated in Table 1. Rinse the containers twice with *water* and then fill completely with a 4 per cent v/v solution of *hydrofluoric acid* and allow to stand at room temperature for ten minutes. Empty the containers and rinse carefully five times with *water*. Carry out the procedure described under *Hydrolytic resistance*. Compare the results with the limits of values given in Table 2. For Type I glass the values obtained with the hydrofluoric acid-treated containers are closely similar to those stated in the Table for Type I or Type II glass; for Type II glass the values obtained with the hydrofluoric acid-treated containers greatly exceed those given in the

TABLE 1

Normal Capacity of Container	Number of Containers to be used	Volume of Test Solution to be used for Titration
5 ml or less	at least 10	50.0 ml
More than 5 ml, up to 30 ml	at least 5	50.0 ml
More than 30 ml	at least 3	100.0 ml

TABLE 2

Capacity of Container (corresponding to 90 per cent average overflow volume)	Volume of *0.01N hydrochloric acid* per 100 ml of Test Solution (ml)	
	Type I or II glass	Type III glass
Not more than 1 ml	2.0	20.00
More than 1 ml but not more than 2 ml	1.8	17.6
More than 2 ml but not more than 5 ml	1.3	13.2
More than 5 ml but not more than 10 ml	1.0	10.2
More than 10 ml but not more than 20 ml	0.80	8.1
More than 20 ml but not more than 50 ml	0.60	6.1
More than 50 ml but not more than 100 ml	0.50	4.8
More than 100 ml but not more than 200 ml	0.40	3.8
More than 200 ml but not more than 500 ml	0.30	2.9
More than 500 ml	0.20	2.2

Table for Type I or Type II glass and are nearer to those given for Type III glass.

Arsenic : Glass ampoules should comply with the following test for arsenic. Carry out the test on ampoules the inner and outer surfaces of which are washed five times with freshly distilled *water*.

Hydrazine-molybdate reagent : Dissolve 0.1 g of *ammonium molybdate* in 10 ml of *water* containing 1.5 ml of *sulphuric acid*, dilute to about 90 ml with *water*, add 1 ml of a 0.15 per cent w/v solution of *hydrazine sulphate* and sufficient *water* to produce 100 ml.

Method : Prepare a test solution as described in the test for *hydrolytic resistance* from an adequate number of ampoules to produce 50 ml. Pipette 10 ml of the test solution from the combined contents of all the ampoules into a flask, add 10 ml of *nitric acid AsT* and evaporate to dryness on a water-bath. Dry the residue in an oven at 130° for thirty minutes. Cool, add to the residue 10.0 ml of *hydrazine-molybdate reagent*, swirl to dissolve and heat under reflux on a water-bath for twenty minutes. Cool to room temperature. Determine the *extinction* of the resulting solution at the maximum at about 840 nm, Appendix 5.15 A, using 10.0 ml of *hydrazine-molybdate reagent* as the blank. The *extinction* of the test solution does not exceed the *extinction* obtained by repeating the determination using 0.1 ml of *dilute arsenic solution AsT* in place of the test solution.

The limit of arsenic, As is 0.1 part per million.

B. PLASTIC CONTAINERS

The following tests are based on the extraction of the plastic material and hence it is important that the designated amount of the plastic is used and the specified surface area should be available for extraction at the designated temperature.

I. Physico-chemical Tests

Preparation of sample – From a homogeneous plastic specimen, use a portion, for each 20.0 ml of *purified water*, equivalent to 120 cm^2 when the thickness is 500 µm or less, or 60 cm^2 when the thickness is greater than 500 µm; subdivide into strips approximately 3 mm in width and as near to 5 cm in length as is practical using clean cutting devices, previously cleaned with *acetone* and *methylene chloride*. Transfer the subdivided *sample* to a glass-stoppered, 250-ml graduated cylinder of Type I glass, and add about 150 ml of *purified water*. Agitate for about 30 seconds, drain off and discard the liquid, and repeat with a second washing.

Transfer the prepared *sample* to a suitable extracting flask, and add the required amount of *purified water*. Extract by heating in an oven at 70° ± 1° for twenty-four hours. Cool, but not below 22°. Decant the extract immediately into a suitably cleaned container, and seal. Prepare a blank omitting the sample. Carry out the following tests on the extract:

(a) Non-volatile matter : Transfer, in suitable portions, 50.0 ml of the extract to a suitable tared crucible, previously cleaned with dilute acid, and evaporate on a water-bath. Dry at 105° for one hour; the residue does not weigh more than 15 mg.

(b) Sulphated ash : Determine the *sulphated ash*, Appendix 3.2.7, using the residue obtained in the test for *non-volatile matter*; the residue on ignition weighs not more than 5 mg.

(c) Heavy metals : Pipette 20 ml of the extract, filtered if necessary, into one of two matched *Nessler cylinders*. Adjust with *N acetic acid* or *6N ammonia* to a pH between 3.0 and 4.0, using short-range pH paper as external indicator, dilute with *water* to about 35 ml, and mix.

Into the second *Nessler cylinder* pipette 2 ml of *standard lead solution* and add 20 ml of *purified water*; adjust the pH to between 3.0 and 4.0 with *N acetic acid* or *6N ammonia* and short-range pH paper as external indicator, dilute with *water* to about 35 ml and mix.

Add 10 ml of freshly prepared *hydrogen sulphide solution* to each cylinder, dilute with *water* to 50 ml, and mix; any brown colour produced within ten minutes in the cylinder containing the extract does not exceed that in the cylinder containing the *standard lead solution*.

(d) Buffering capacity : Pipette a 20-ml portion of the extract of the prepared *sample*, prior to decanting the extract from the extraction container, into a suitable container. Titrate potentiometrically to a pH of 7.0, using either *0.01N hydrochloric acid* or *0.01N sodium hydroxide*, as required. Treat a 20-ml portion of the blank in the same manner. If the same titrant was required for both samples extract and blank, the difference between the two volumes is not greater than 10.0 ml, and if acid was required for either the sample extract or the blank and alkali for the other, the total of the two volumes required is not greater than 10.0 ml.

II. Biological Tests

Place two properly prepared samples (*see* Preparation of sample) to be tested in separate extraction flasks, and add to each 20-ml of *sodium chloride injection*. Also prepare one 20-ml blank of *sodium chloride injection*. Extract by heating in an autoclave at 121° for 60 minutes, in an oven at 70° for 24 hours, or at 50° for 72 hours. Allow adequate time for the liquid within the container to reach the extraction temperature. Cool to room temperature but not below 22°, shake vigorously and decant each extract into a dry sterile container. Store the extracts at a temperature between 22° and 30° and do not use for tests after 24 hours.

Inject intravenously 1.0 ml of each extract and of the blank at the rate of 0.1 ml per second into each of five heal-

thy, not previously used albino mice, weighing between 17 and 22 g. Observe the animals immediately after injection, again 4 hours after injection, and then not earlier than 24, 48 and 72 hours, respectively, after injection. The sample passes the test if, during the observation period none of the animals treated with the sample extracts shows a significantly greater reaction than the animals treated with the blank. If any animals treated with the sample extract shows slight signs of toxicity, and not more than one animal shows gross symptoms of toxicity or dies, repeat the test using ten animals. On the repeated test, the requirements of the test are met if none of the animals treated with the sample extract shows a significantly greater reaction than that observed in the animals treated with the blank.

C. TRANSFUSION AND INFUSION ASSEMBLIES

Disposable transfusion and infusion assemblies comply with the following requirements:

(1) Sterility : Proceed as described under *Sterile Devices* under *tests for sterility*, Appendix 4.6.

(2) Pyrogens : Select ten assemblies representative of the production of each working day and through the tubing of each pass a separate 40-ml portion of sterile, pyrogen-free *saline solution* at a flow rate of approximately 10 ml per minute. The pooled effluent meets the requirements of the *test for pyrogens*, Appendix 2.36, the test dose being 10 ml per kg of body weight.

(3) Safety : Select one assembly representative of the production of each working day. Fill the assembly as completely as practicable with sterile *saline solution*, clamp the ends securely to retain the solution and immerse the filled assembly completely in water. Heat the water at not less than 85° for one hour. Drain the contents of the assembly, and dilute with sterile *saline solution* to 250 ml. Inject intravenously 0.5 ml of this solution into each of five healthy mice weighing between 17 and 22 g; at the end of four, twenty-four and forty-eight hours the animals show no discernible signs of toxicity. If any of the animals shows gross signs of toxicity or dies, repeat the test with another five healthy mice weighing between 19 and 21 g; all the animals survive for forty-eight hours.

Other requirements : Parts of the assemblies that are made of plastic meet the requirements stated under **Plastic Containers**.

3.3.29 Determination of Cineole

Weigh 3.00 g of the substance being examined, recently dried with *anhydrous sodium sulphate*, into a dry test-tube and add 2.1 g of melted *o-cresol*. Place the tube in the apparatus for the *determination of congealing range or temperature*, Appendix 5.5, and allow to cool, with constant stirring. When crystallisation takes place, there is a small rise in temperature; note the highest temperature reached (t_1).

Remelt the mixture on a water-bath ensuring that the temperature does not exceed t_1 by more than 5°, and place the tube in the apparatus for the determination of freezing point maintained at a temperature 5° below t_1. When recrystallisation takes place, or when the temperature of the mixture has fallen 3° below t_1, stir continuously; note the highest temperature at which the mixture freezes (t_2). Repeat the operation until the two highest values obtained for t_2 do not differ by more than 0.2°. If supercooling occurs, induce, crystallisation by the addition of a small crystal of a complex consisting of 3.00 g of *cineole* and 2.10 g of *o-cresol*. If t_2 is below 27.4°, repeat the determination after the addition of 5.10 g of the complex.

Determine the percentage w/w of cineole corresponding to the freezing point (t_2) from the Table 1, obtaining intermediate values by interpolation. If 5.10 g of the *cineole/o-cresol* complex was added, calculate the percentage w/w of cineole from the expression $2(A-50)$, where A is the value corresponding to a freezing point of t_2 taken from the Table 1.

TABLE 1

t_2°	Cineole per cent w/w	t_2°	Cineole per cent w/w
24	45.5	40	67.0
25	47.0	41	68.5
26	48.5	42	70.0
27	49.5	43	72.5
28	50.5	44	74.0
29	52.0	45	76.0
30	53.5	46	78.0
31	54.5	47	80.0
32	56.0	48	82.0
33	57.0	49	84.0
34	58.5	50	86.0
35	60.0	51	88.5
36	61.0	52	91.0
37	62.5	53	93.5
38	63.5	54	96.0
39	65.0	55	99.0

4. Microbiological Tests and Assays

4.1 MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

The inhibition of microbial growth under standardised conditions may be utilised for demonstrating the therapeutic efficacy of antibiotics. Any subtle change in the antibiotic molecule which may not be detected by chemical methods will be revealed by a reduction in the antimicrobial activity and hence microbiological assays are very useful for resolving doubts regarding possible loss of potency of antibiotics and their preparations.

The microbiological assay is based upon a comparison of the inhibition of growth of bacteria by measured concentrations of the antibiotics to be examined with that produced by known concentrations of a standard preparation of the antibiotic having a known activity. Two general methods are usually employed, the cylinder-plate (or cup-plate) method and the turbidimetric (or tube assay) method.

The assay method used must be proven, valid by statistical analysis of the actual data constructing the assay, i.e. the dose-response lines produced by the standard and the preparation to be examined must be shown to be linear and parallel within a given probability level.

Standard Preparation and Units of Activity

A Standard Preparation is an authentic sample of the appropriate antibiotic for which the potency has been precisely determined by reference to the appropriate international standard. The potency of the standard preparation may be expressed in International Units or in μg per mg of the pure antibiotic.

The Standard Preparations for India are maintained at the Central Drugs Laboratory, Calcutta. A Unit referred to in the official assays and tests, is the specific activity contained in such an amount of the respective Standard Preparation as is indicated by the Ministry of Health & Family Welfare, Government of India, from time to time.

A Standard Preparation may be replaced by a working standard prepared by any laboratory which should be compared at definite intervals under varying conditions with the standard.

A description of the Standard Preparation of the antibiotics and their activity is given in Table 1.

Test Organisms and Inoculum

The test organism recommended for each antibiotic and method of preparation of microbial suspension is listed in Table 5. If suspensions are prepared by the methods described under Table 5, growth characteristics are sufficiently uniform so that the inoculum for assays can be adequately determined by the following test trials:

For Method A : After the suspension is prepared, as given under Table 5, add different volumes of it to each of several different flasks containing 100 ml of the medium specified in Table 4 (the volume of suspension suggested in Table 4 may be used as a guide). Using these inocula, prepare inoculated plates as described for the specific antibiotic assay. Fill each cylinder with the median concentration of the antibiotic (Table 4) and then incubate the plates. After incubation, examine and measure the zones of inhibition. The volume of suspension that produces the optimum zones of inhibition with respect to both clarity and diameter determines the inoculum to be used for the assay.

For Method B : Proceed as described for Method A and using the several inocula, proceed as described for the specific antibiotic assay, but run only the high and low concentrations of the standard response curve. After incubation, read the extinctions of the appropriate tubes. Determine which inoculum produces the best response between the low and high antibiotic concentrations and use this inoculum for the assay.

Apparatus

All equipment is to be thoroughly cleaned before and after each use. Glassware for holding and transferring test organisms is sterilized by dry heat, or by steam.

Temperature control : Thermostatic control is required in several stages of a microbial assay, when culturing a micro-organism and preparing its inoculum, and during incubation in a plate assay. Closer control of the temperature is imperative during incubation in a tube assay, and may be achieved by either circulated air or water, the greater heat capacity of water lending it some advantage over circulating air.

Spectrophotometer : Measuring transmittance within a fairly narrow frequency band requires a suitable spectrophotometer in which the wavelength of the light source can be varied or restricted by the use of a 580-nm filter for preparing inocula of the required density, or with a 530-nm filter for reading the extinction in a tube assay. For the latter purpose, the instrument may be arranged to accept the tube in which incubation takes place, to accept a modified cell fitted with a drain that facilitates rapid change of content, or preferably fixed with a flow-through cell for a

TABLE 1.—Standard Preparations

Antibiotic	Standard Preparation	Activity (Units/μg per mg)	mg Containing 1 Unit
Amphotericin B	Amphotericin B	940 Units	0.001064
Ampicillin	Ampicillin	850 μg	0.0011764
Bacitracin	Zinc Bacitracin	74 Units	0.01351
Carbenicillin sodium	Carbenicillin sodium, monohydrate	881 μg	0.001135
Cephalexin	Cephalexin	933 μg	0.0010707
Chloramphenicol	Chloramphenicol	1000 μg	0.001
Demethylchlortetracycline	Demethylchlortetracycline hydrochloride	1000 μg	0.001
Doxycycline	Doxycycline hydrochloride; hemiethanolate, hemihydrate	870 μg	0.0011494
Erythromycin	Erythromycin dihydrate	950 μg	0.001053
Gentamicin	Gentamicin sulphate	641 μg	0.00156
Kanamycin	Kanamycin sulphate	812 Units	0.001232
Lincomycin	Lincomycin hydrochloride	865 μg	0.001156
Neomycin	Neomycin sulphate	680 μg	0.00147
Novobiocin	Novobiocin	970 μg	0.001031
Nystatin	Nystatin	3000 Units	0.000333
Oxytetracycline	Oxytetracycline dihydrate	880 μg	0.0011364
Rifampicin	Rifampicin sodium	887 μg	0.001127
Streptomycin	Streptomycin sulphate	780 μg	0.001282
Tetracycline	Tetracycline hydrochloride	982 μg	0.00101833

continuous flow-through analysis; set the instrument at zero extinction with clear, uninoculated broth prepared as specified for the particular antibiotic, including the same amount of test solution and formaldehyde as found in each sample.

Cylinder-plate assay receptacles : Use rectangular glass trays or glass or plastic petri-dishes (approximately 20 × 100 mm) having covers of suitable material. For assay cylinders, use cylinders with the following dimensions, each dimension having a tolerance of ± 0.1 mm; outside diameter 8 mm; inside diameter 6 mm and length 10 mm; and made of glass, porcelain, aluminium or stainless steel. Instead of cylinders, holes 5 to 8 mm in diameter may be bored in the medium with a sterile borer, or paper discs of suitable quality paper may be used. Carefully clean cylinders to remove all residues. An occasional acid-bath, e.g. with about *2N nitric acid* or with *chromic acid solution* is needed.

Turbidimetric assay receptacles : For assay tubes, use glass or plastic test-tubes, e.g. 16 mm × 125 mm or 18 mm × 150 mm that are relatively uniform in length, diameter, and thickness and substantially free from surface blemishes and scratches. Cleanse thoroughly, to remove all antibiotic residues and traces of cleaning solution, and sterilize tubes that have been used previously before subsequent use.

Assay Designs

Microbial assays gain markedly in precision by the segregation of relatively large sources of potential error and bias through suitable experimental designs. In a cylinder-plate assay, the essential comparisons are restricted to relationships between zone diameter measurements within plates, exclusive of the variation between plates in their preparation and subsequent handling. To conduct a turbidimetric assay so that the differences in observed turbidity will reflect the differences in the antibiotic concentration requires both greater uniformity in the environment created for the tubes through closer thermostatic control of the incubator and the avoidance of systematic bias by a random placement of replicate tubes in separate tube racks, each rack containing one complete set of treat-

ments. The essential comparisons are then restricted to relationships between the observed turbidities within racks.

Within these restrictions, two alternative designs are recommended; i.e. a 3-level (or 2-level) factorial assay, or a 1-level assay with a standard curve. For a factorial assay, prepare solutions of 3 or 2 corresponding test dilutions for both the standard and the unknowns on the day of the assay, as described under Preparation of the Standard and Preparation of the Sample. For a 1-level assay with a standard curve, prepare instead solutions of 5 test dilutions of the standard and a solution of a single median test level of the unknown as described in the same sections. Consider an assay as preliminary if its computed potency with either design is less than 60 per cent or more than 150 per cent of that assumed in preparing the stock solution of the unknown. In such a case, adjust its assumed potency accordingly and repeat the assay.

Microbial determinations of potency are subject to inter-assay as well as intra-assay variables, so that two or more independent assays are required for a reliable estimate of the potency of a given assay preparation or unknown. Starting with separately prepared stock solutions and test dilutions of both the standard and the unknown, repeat the assay of a given unknown on a different day. If the estimated potency of the second assay differs significantly, as indicated by the calculated standard error, from that of the first, conduct one or more additional assays. The combined result of a series of smaller, independent assays spread over a number of days is a more reliable estimate of potency than that from a single large assay with the same total number of plates or tubes.

Methods

The microbiological assay of antibiotics may be carried out by Method A or Method B.

A. CYLINDER-PLATE OR CUP-PLATE METHOD

This method depends on the diffusion of an antibiotic from a vertical cylinder or a cavity through the solidified agar layer of a petri-dish or plate to an extent such that growth of the added micro-organism is prevented entirely in a circular area or "zone" around the cylinder or cavity containing a solution of the antibiotic.

Inoculate a previously liquefied medium appropriate to the assay (Tables 3 and 4) with the requisite quantity of suspension of the micro-organism (*see* section entitled Test Organism and Inoculum); and the suspension to the medium at a temperature between 40° and 50° and immediately pour the inoculated medium into petri-dishes or large rectangular plates to give a depth of 3 to 4 mm (1 to 2 mm for nystatin). Ensure that the layers of medium are uniform in thickness, by placing the dishes or plates on a levelled surface.

The prepared dishes or plates must be stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes or plates are used and that the surface of the agar layer is dry at the time of use.

Using the appropriate buffer solutions indicated in Tables 2 & 4, prepare solutions of known concentration of the Standard Preparation and solutions of the corresponding assumed concentrations of the antibiotic to be examined. Where directions have been given in the indivi-

TABLE 2 – Buffer Solutions

Buffer solutions are prepared by dissolving the following quantities of *dipotassium hydrogen phosphate* and *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml after adjusting the pH with *18N phosphoric acid* or *10N potassium hydroxide*

Buffer Number	Dipotassium Hydrogen Phosphate K_2HPO_4 (g)	Potassium Dihydrogen Phosphate KH_2PO_4 (g)	pH Adjusted to
1	2.0	8.0	6.0 ± 0.1
2	16.73	0.593	8.0 ± 0.1
3	–	13.61	4.5 ± 0.1
4	20.0	80.00	6.0 ± 0.1
5	35.0	–	10.5 ± 0.1 (after addition of 2 ml of *10N potassium hydroxide*)

TABLE 3 – Composition of Media

Media are prepared by dissolving the following quantities (in g) of the specified ingredients in sufficient *water* to produce 1000 ml of Medium

Ingredients	Medium A	Medium B	Medium C	Medium D	Medium E	Medium F	Medium G	Medium H
Peptone	6.0	6.0	5.0	6.0	6.0	6.0	9.4	–
Pancreatic digest of casein	4.0	–	–	4.0	–	–	–	17.0
Yeast extract	3.0	3.0	1.5	3.0	3.0	3.0	4.7	–
Beef extract	1.5	1.5	1.5	1.5	1.5	1.5	2.4	–
Dextrose	1.0	–	1.0	1.0	–	–	10.0	2.5
Papaic digest of soybean	–	–	–	–	–	–	–	3.0
Agar	15.0	15.0	–	15.0	15.0	15.0	23.5	20.0
Sodium chloride	–	–	3.5	–	–	–	10.0	5.0
Dipotassium hydrogen phosphate	–	–	3.68	–	–	–	–	2.5
Potassium dihydrogen phosphate	–	–	1.32	–	–	–	–	–
Final pH (after sterilization)	6.5–6.6	6.5–6.6	6.95–7.05	7.8–8.0	7.8–8.0	5.8–6.0	6.0–6.2	7.2–7.3

dual monographs for preparing the solutions, these should be followed and further dilutions done with buffer solution as indicated in Table 4. Apply the solutions to the surface of the solid medium in sterile cylinders or in cavities prepared in the agar. The volume of solution added to each cylinder or cavity must be uniform and sufficient almost to fill the holes when these are used. When paper discs are used these should be sterilised by exposure of both sides under a sterilising lamp and then impregnated with the standard solutions or the test solutions and placed on the surface of the medium. When petri dishes are used, arrange the solutions of the Standard Preparation and the antibiotic to be examined on each dish so that they alternate around the dish and so that the highest concentrations of standard and test preparations are not adjacent. When plates are used, place the solutions in a Latin Square design, if the plate is a square, or if it is not, in a randomised block design. The same random design should not be used repeatedly.

Leave the dishes or plates standing for one to four hours, at room temperature or at 4°, as appropriate, as a period of pre-incubation diffusion to minimise the effects of variation in time between the application of the different solutions. Incubate them for about eighteen hours at the temperature indicated in Table 4. Accurately measure the diameters or areas of the circular inhibition zones and calculate the results.

Selection of the assay design should be based on the requirements stated in the individual monograph. Some of the usual assay designs are as follows:

(a) One-level assay with standard curve

Standard solution : Dissolve an accurately weighed quantity of the Standard Preparation of the antibiotic, previously dried where necessary, in the solvent specified in Table 4, and then dilute to the required concentration as indicated, to give the stock solution. Store in a refrigerator and use within the period indicated. On the day of the assay, prepare from the stock solution, 5 dilutions (solutions S_1 to S_5) representing five test levels of the standard and increasing stepwise in the ratio of 4:5. Use the diluent specified in Table 4 and a sequence such that the middle or median has the concentration given in the last column of the Table.

Sample solution : From the information available for the antibiotic preparation which is being examined (called the unknown) assign to it an assumed potency per unit weight or volume and on this assumption prepare on the day of the assay a stock solution with the same solvent as used for the standard. Prepare from this stock solution a dilution to a concentration equal to the median level of the standard to give the sample solution.

Method : For preparing the standard curve, use a total

TABLE 4

Antibiotic	Test Organism	ATCC No.[•]	NCTC No.[••]	Suggested ml of Suspension per 100 ml of Media	Assay Method	Medium
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Amphotericin B	*Saccharomyces cerevisiae*	9763	10716	1.0	A	G
Ampicillin	*Micrococcus luteus*	9341	8553 (NC1B)[†]	0.5	A	D
Bacitracin	*Micrococcus luteus*	10240	7743	0.3	A	A
Carbenicillin sodium	*Pseudomonas aeruginosa*	25619	–	0.5	A	H
Cephalexin	*Staphylococcus aureus*	29737	–	0.05	A	A
Chloramphenicol	*Escherichia coli*	10536	–	0.7	B	C
Demethylchlortetracycline	*Staphylococcus aureus*	29737	–	0.1	B	C
Doxycycline	*Staphylococcus aureus*	29737	–	0.1	B	C
Erythromycin	*Micrococcus luteus*	9341	8553 (NC1B)[†]		A	D
Gentamicin	*Staphylococcus epidermides*	12228	–	•	A	D
Kanamycin sulphate	*Bacillus pumilus*	14884	8241	•	A	D
Kanamycin sulphate	*Staphylococcus aureus*	29737	–	0.2	B	C
Kanamycin B	*Bacillus subtilis*	6633	8236	•	A	E

contd

TABLE 4

Working Standard Stock Solution				Test Doses			
Initial solvent & initial concentration	Further diluent	Concentration per ml	Use within	Final diluent	Media concentration per ml	For two level factorial assays per ml	Incubation temperature °C
(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
–	Dimethyl Sulphoxide	1000 Units	Same day	B5	1.0 Unit	1.0 to 4.0 Units	30
–	Water	100 µg	7 days	B2	0.1 µg	–	32 – 35
–	B1	100 Units	Same day	B1	1.0 Unit	1.0 to 4.0 Units	32 – 35
–	B1	1 mg	14 days	B1	20 µg	–	37
–	B1	1 mg	7 days	B1	20 µg	–	32 – 35
Ethyl alcohol (10 mg/ml)	B1	1 mg	30 days	B1	2.5 µg	–	35 – 37
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	4 days	Water	0.1 µg	–	35 – 37
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	5 days	B3	0.1 µg	–	35 – 37
Mthyl alcohol (10 mg/ml)	B2	1 mg	14 days	B2	1.0 µg	–	35 – 37
–	B2	1 mg	30 days	B2	0.1 µg	–	35 – 37
–	B2	800 Units	30 days	B2	0.8 Unit	5.0 to 20.0 Units	37 – 39
Water	Water	1000 Units	30 days	Water	10 Units	–	32 – 35
–	B2	1000 Units	30 days	B2	1.0 Unit	–	32 – 35

TABLE 4 – *contd*

TABLE 4 – *contd*

Antibiotic	Test Organism	ATCC ● No.	NCTC ●● No.	Suggested ml of Suspension per 100 ml of Media	Assay Method	Medium
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Lincomycin Hydrochloride	*Micrococcus luteus*	9341	8553 (NC1B)†	1.5	A	D
Neomycin sulphate	*Staphylococcus epidermides*	12228	8853 (NC1B)†	0.4	A	D
Novobiocin sodium	*Staphylococcus epidermides*	12228	8853 (NC1B)†	••	A	A
Nystatin	*Saccharomyces cerevisiae*	2601	10716	1.0	A	G
Oxytetracycline	*Bacillus cereus* var. *mycoides*	11778	10320	•	A	F
Oxytetracycline	*Staphylococcus aureus*	29737	–	0.1	B	C
Rifampicin	*Bacillus subtilis*	6633	8236	•	A	B
Streptomycin sulphate	*Bacillus subtilis*	6633	8236	•	A	E
Streptomycin sulphate	*Klebsiella pneumoniae*	10031	9111 (NC1B)	0.1	B	C
Tetracycline	*Bacillus cereus*	11778	10320	•	A	F
Tetracycline	*Staphylococcus aureus*	6538P	–	0.1	B	C

● American Type Culture Collection, 21301 Parklawn Drive, Rock Ville, Md 20852.

●● National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW95HT, England.

† National Collection of Industrial Bacteria; Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen Ab 98 DC Scotland

contd

TABLE 4 – *contd*

Working Standard Stock Solution				Test Doses			
Initial solvent & initial concentration	Further diluent	Concentration per ml	Use within	Final diluent	Media concentration per ml	For two level factorial assays per ml	Incubation temperature °C
(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	Water	1 mg	30 days	Water	2.0 µg	–	32 – 35
–	B_2	1 mg	14 days	B2	1.0 µg	–	35 – 37
Ethyl alcohol (10 mg/ml)	B1	1 mg	5 days	B1	0.5 µg	–	32 – 35
–	Dimethyl formamide	1000 Units	Same day	B4	20 Units††	–	30
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	4 days	B3	2.5 µg	–	32 – 35
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	4 days	Water	0.24 µg	–	35 – 37
–	Methyl alcohol	1 mg	1 day	B1	5.0 µg	–	32 – 35
Water	Water	1 mg	30 days	Water	1.0 µg	5.0 to 20.0 µg	32 – 35
Water	Water	1 mg	30 days	Water	30 µg	–	35 – 37
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	1 day	B3	2.5 µg	–	32 – 35
0.1 N hydrochloric acid	0.1 N hydrochloric acid	1 mg	4 days	Water	0.24 µg	–	35 – 37

x "B" Denotes "buffer solution and the number following refers to the buffer solution detailed in Table 2.

• Determine dilution factor and inoculum amount by test plate method.

•• Determine dilution factor and inoculum amount by trial tube method.

†† Dilute aliquots of the working standard stock solution with *dimethyl formamide* to give 256 Units, 320 Units, 400 Units, 500 Units and 624 Units per ml.

TABLE 5

Test Organism	ATCC No.	Method of Preparation	Medium used for		Incubation Period for		Incubation Temperature for Slant & Roux bottle °C	Suggested Dilution Factor	Storage Period for Suspension at a Temperature not Exceeding 4°C
			Slant	Roux bottle	Slant	Roux bottle			
Bacillus cereus var *mycoides*	11778	2	A	A*	24 hours	1 week	35 - 37	**	6 months
Bacillus pumilus	14884	2	A	A	24 hours	1 week	35 - 37	**	6 months
Bacillus subtilis	6633	2	A	A*	24 hours	1 week	35 - 37	**	6 months
Escherichia coli	10536	1	A	A	24 hours	24 hours	32 - 35	1 : 20	2 weeks
Klebsiella pneumoniae	10031	1	A	A	24 hours	24 hours	32 - 35	1 : 25	1 week
Micrococcus luteus	9341	1	A	A	24 hours	24 hours	32 - 35	1 : 30	2 weeks
Micrococcus luteus	10240	1	A	A	24 hours	24 hours	32 - 35	1 : 35	4 weeks
Pseudomonas aeruginosa	25619	1	A	A	24 hours	24 hours	32 - 35	1 : 25	2 weeks
Saccharomyces cerevisiae	9763	3	G	–	24 hours	24 hours	32 - 35	1 : 30	4 weeks
Saccharomyces cerevisiae	2601	3	G	–	24 hours	24 hours	32 - 35	1 : 30	4 weeks
Staphylococcus aureus	29737	1	A	A	24 hours	24 hours	32 - 35	1 : 20	1 week
Staphylococcus epidermides	12228	1	A	A	24 hours	24 hours	32 - 35	1 : 40	2 weeks

* Use Medium A containing 300 mg of $MnSO_4.H_2O$ per litre.

** Determine dilution factor of suspension by test plate method or trials in broth.

Methods of Preparation of Test Organism Suspension:

(1) Maintain the test organism on slants of Medium A and transfer to a fresh slant once a week. Incubate the slants at the temperature indicated above for 24 hours. Using 3 ml of *saline solution*, wash the organism from the agar slant onto a large agar surface of Medium A, such as a Roux bottle containing 250 ml of agar. Incubate for 24 hours at the appropriate temperature. Wash the growth from the nutrient surface using 50 ml of *saline solution*. Store the test organism under refrigeration. Determine the dilution factor which will give 25% light transmission at 530 nm. Determine the amount of suspension to be added to each 100 ml of agar or nutrient broth by use of test plates or test broth. Store the suspension under refrigeration.

(2) Proceed as described in Method (1) but incubate the Roux bottle for 5 days. Centrifuge and decant the supernatant liquid. Resuspend the sediment with 50 – 70 ml of *saline solution* and heat the suspension for 30 minutes at 70°C. Wash the spore suspension three times with 50 – 70 ml of *saline solution*. Resuspend in 50 – 70 ml of *saline solution* and heat-shock again for 30 minutes. Use test plates to determine amount of the suspension required for 100 ml agar. Maintain suspension under refrigeration.

(3) Maintain test organism on 10 ml agar slants of Medium G. Incubate at 32° – 35°C for 24 hours. Inoculate 100 ml of nutrient broth. Incubate for 16 – 18 hours at 37°C and proceed as described in Method (1).

of 12 petri-dishes or plates to accommodate 72 cylinders or cavities. A set of 3 plates (18 cylinders or cavities) is used for each dilution. On each of the three plates of a set fill alternate cylinders or cavities with solution S_3 (representing the median concentration of the standard solution) and each of the remaining 9 cylinders or cavities with one of the other 4 dilutions of the standard solution. Repeat the process for the other 3 dilutions of the standard solution. For each unknown preparation use a set of three plates (18 cylinders or cavities) and fill alternate cylinders or cavities with the sample solution and each of the remaining 9 cylinders of cavities with solution S_3.

Incubate the plates for about 18 hours at the specified temperature and measure the diameters of the zones of inhibition.

Estimation of potency : Average the readings of solution S_3 and the readings of the concentration tested on each set of three plates, and average also all 36 readings of solution S_3. The average of the 36 readings of solution S_3 is the correction point for the curve. Correct the average value obtained for each concentration (S_1, S_2, S_4 and S_5) to the figure it would be if the reading for solution S_3 for that set of three plates were the same as the correction point. Thus, in correcting the value obtained with any concentration, say S_1, if the average of 36 readings of S_3 is, for example, 18.0 mm, and the average of the S_3 concentrations on one set of 3 plates is 17.8 mm, the correction is +0.2 mm. If the average reading of S_1 is 16.0 mm, the corrected reading of S_1 is 16.2 mm. Plot these corrected values including the average of the 36 readings for solutions S_3 on two-cycle semilog paper, using the concentrations in Units or μg per ml (as the ordinate logarithmic scale) and the diameter of the zones of inhibition as the abscissa. Draw the straight response line either through these points by inspection or through the points plotted for highest and lowest zone diameters obtained by means of the following equation:

$$L = \frac{3a+2b+c-e}{5} \qquad H = \frac{3c+2d+c-a}{5}$$

where

L = the calculated zone diameter for the lowest concentration of the standard curve response line.

H = the calculated zone diameter for the highest concentration of the standard curve response line.

C = average zone diameter of 36 readings of the reference point standard solution.

a, b, d, e = corrected average values for the other standard solutions, lowest to highest concentration, respectively.

Average the zone diameters for the sample solution and for solution S_3 on the plates used for the sample solution. If the sample gives a larger average zone size than the average of the standard (solution S_3), add the difference between them to the zone size of solution S_3 of the standard response line. If the average sample zone size is smaller than the standard values, subtract the difference between them from the zone size of solution S_3 of the standard response line. From the response line read the concentration corresponding to these corrected values of zone sizes. From the dilution factors the potency of the sample may be calculated.

(b) Two-level factorial assay

Prepare parallel dilutions containing 2 levels of both the standard (S_1 and S_2) and the unknown (U_1 and U_2). On each of four or more plates, fill each of its four cylinders or cavities with a different test dilution alternating standard and unknown. Keep the plates at room temperature for about one to four hours and then incubate them for about eighteen hours at the specified temperature and measure the diameters of the zones of inhibition.

Estimation of potency : Sum the diameters of the zones of each dilution and calculate the per cent potency of the sample (in terms of the standard) from the following formula:

$$\text{per cent potency} = \text{Antilog } (2.0 + a \log I)$$

$$\text{where } a^* = \frac{(U_1 + U_2) - (S_1 + S_2)}{(U_1 - U_2) + (S_1 - S_2)}$$

U_1 and U_2 are the sums of the zone diameters with solutions of the unknown of high and low levels.

S_1 and S_2 are the sums of the zone diameters with solutions of the standard of high and low levels.

I = ratio of dilutions

*a, May have a positive or negative value and should be used algebraically.

If the potency of the sample is lower than 60 per cent or greater than 150 per cent of the standard, the assay is invalid and should be repeated using higher or lower dilutions of the sample solution.

The potency of the sample may be calculated from the formula:

$$\frac{\text{per cent potency} \times \text{assumed potency of the sample}}{100}$$

(c) Other designs

(1) Factorial assay containing parallel dilutions of three test levels of standard and the unknown.

(2) Factorial assay using two test levels of standard and two test levels of two different unknowns.

B. TURBIDIMETRIC OR TUBE ASSAY METHOD

This method depends upon the growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favourable to its rapid growth in the

absence of the antibiotic. The method has the advantage of a shorter incubation period for the growth of the test organism (usually 3 to 4 hours) but the presence of solvent residues or other inhibitory substances affects this assay more than the cylinder-plate assay and care should be taken to ensure freedom from such substances in the final test solutions. This method is not recommended for cloudy or turbid preparations.

Prepare five different concentrations of the standard solution for preparing the standard curve by diluting the stock solution of the Standard Preparation of the antibiotic. Select the median concentration (Table 4) and dilute the solution of the antibiotic being examined (unknown) to obtain approximately this concentration. Place 1 ml of each concentration of the standard solution and of the sample solution in each of the tubes in duplicate. To each tube add 9 ml of nutrient medium (Table 4) previously seeded with the appropriate test organism (Table 4).

At the same time prepare three control tubes, one containing the inoculated culture medium (culture control), another identical with it but treated immediately with 0.5 ml of *dilute formaldehyde solution* (blank) and a third containing uninoculated culture medium.

Place all the tubes, randomly distributed or in a randomised block arrangement in an incubator or a water-bath and maintain them at the specified temperature (Table 4) for three to four hours. After incubation add 0.5 ml of *dilute formaldehyde solution* to each tube. Measure the growth of the test organism by determining the *extinction* at 530 nm of each of the solutions in the tubes against the blank, Appendix 5.15 A.

Estimation of potency : Plot the average *extinctions* for each concentration of the standard on semi-logarithmic paper with the *extinctions* on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight response line through the points either by inspection or by means of the following equations:

$$L = \frac{3a+2b+c-e}{5} \qquad H = \frac{3a+2d+c-a}{5}$$

where

L = the calculated extinction for the lowest concentration of the standard response line.

H = the calculated extinction for the highest concentration of the standard response line.

a,b,c,d,e = average extinction values for each concentration of the standard response line lowest to highest respectively.

Plot the values obtained for L and H and connect the points. Average the extinctions for the sample and read the antibiotic concentration from the standard response line. Multiply the concentration by the appropriate dilution factors to obtain the antibiotic content of the sample.

Precision of Microbiological Assays

The fiducial limits of error of the estimated potency, should be not less than 95% and not more than 105% of the estimated potency, unless stated otherwise in the individual monograph.

This degree of precision is the minimum acceptable for determining that the final product complies with the official requirements and may be inadequate for those deciding, for example, the potency which should be stated on the label or used as the basis for calculating the quantity of an antibiotic to be incorporated in a preparation. In such circumstances, assays of greater precision may be desirable, with, for instance, fiducial limits of error of the order of 98 to 102 per cent. With this degree of precision, the lower fiducial limit lies close to the estimated potency. By using this limit, instead of the estimated potency, to assign a potency to the antibiotic either for labelling or for calculating the quantity to be included in a preparation, there is less likelihood of the final preparation subsequently failing to comply with the official requirements for potency.

4.2 ASSAY OF CALCIUM PANTOTHENATE

Reagents

Standard stock solution of calcium pantothenate : Weigh accurately 50 mg of *calcium pantothenate R.S.*, previously dried at 105° for three hours and stored in the dark over phosphorus pentoxide, taking care to protect from absorption of moisture during the weighing. Dissolve in about 500 ml of *water*, add 10 ml of *0.2N acetic acid*, 100 ml of a 1.6 per cent w/v solution of *sodium acetate* and sufficient *water* to produce 1000.0 ml. Each ml of this solution contains 50 µg of calcium pantothenate. Store under *toluene* in a refrigerator.

Standard solution : On the day of the assay, dilute an accurately measured volume of *standard stock solution of calcium pantothenate* with sufficient *water* to produce a solution containing between 0.01 µg and 0.04 µg of calcium pantothenate per ml.

Test solution : Proceed as described in the individual monograph or prepare a solution in *water* such that it contains approximately the equivalent of the calcium pantothenate concentration in the *standard solution*.

Medium solution:

Acid-hydrolyzed Casein Solution	25 ml
Cystine-Tryptophane Solution	25 ml
Polysorbate 80 Solution	0.25 ml
Dextrose, Anhydrous	10 g
Sodium Acetate, Anhydrous	5 g
Adenine-Guanine-Uracil Solution	5 ml

Riboflavin-Thiamine Hydrochloride-Biotin Solution	5 ml
Para-aminobenzoic Acid-Niacin-Pyridoxine Hydrochloride Solution	5 ml
Salt Solution A	5 ml
Salt Solution B	5 ml

Dissolve the anhydrous dextrose and sodium acetate in the solutions previously mixed, and adjust with *1N sodium hydroxide* to a pH of 6.8. Finally, dilute with *water* to 250 ml, and mix.

Acid-hydrolyzed casein solution : Mix 100 g of *vitamin-free casein* with 500 ml of *6N hydrochloric acid*, and reflux the mixture for 8 to 12 hours. Remove the hydrochloric acid from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in *water*, adjust the solution with *N sodium hydroxide* to a pH of 3.5 ± 0.1, and add *water* to make 1000.0 ml. Add 20 g of *activated charcoal*, stir for 1 hour, and filter. Repeat the treatment with *activated charcoal*. Store under *toluene* in a refrigerator at a temperature not below 10°. Filter the solution if a precipitate forms during storage.

Cystine-tryptophane solution : Suspend 4.0 g of L-*cystine* and 1.0 g of L-*tryptophane* (or 2.0 g of D,L-tryptophane) in 700 to 800 ml of *water*, heat to 70° to 80°, and add dilute *hydrochloric acid* (1 in 2) dropwise, with stirring, until the solids are dissolved. Cool, and add *water* to make 1000 ml. Store under *toluene* in a refrigerator at a temperature not below 10°.

Adenine-guanine-uracil solution : Dissolve 200 mg each of *adenine sulphate, guanine hydrochloride*, and *uracil*, with the aid of heat, in 10 ml of *4N hydrochloric acid*, cool, and add water to make 200 ml. Store under *toluene* in a refrigerator.

Polysorbate 80 solution : Dissolve 25 g of *polysorbate 80* in *alcohol* to make 250 ml.

Riboflavin-thiamine hydrochloride-biotin solution : Prepare a solution containing, in each ml, 20µg of riboflavin, 10 µg of thiamine hydrochloride, and 0.04 µg of biotin, by dissolving *riboflavin, thiamine hydrochloride*, and *biotin* in *0.02N acetic acid*. Store, protected from light, under *toluene* in a refrigerator.

Para-aminobenzoic acid-niacin-pyridoxine hydrochloride solution : Prepare a solution in neutral *alcohol (25 per cent)* to contain 10 µg of *para-amino benzoic acid*, 50 µg of *niacin*, and 40 µg of *pyridoxine hydrochloride* in each ml. Store in a refrigerator.

Salt solution A : Dissolve 25 g of *potassium dihydrogen phosphate* and 25 g of *dipotassium hydrogen phosphate* in *water* to make 500 ml. Add 5 drops of *hydrochloric acid*, and store under *toluene*.

Salt solution B : Dissolve 10 g of *magnesium sulphate*, 0.5 g of *sodium chloride*, 0.5 g of *ferrous sulphate*, and 0.5 g of *manganese sulphate* in *water* to make 500 ml. Add 5 drops of *hydrochloric acid*, and store under *toluene*.

Stock culture of organism : Dissolve 2.0 g of water-soluble yeast extract in 100 ml of water, add 500 mg of *dextrose (anhydrous)*, 500 mg of *anhydrous sodium acetate*, and 1.5 g of *agar*, and heat the mixture, with stirring, on a steam bath, until the agar dissolves. Add approximately 10 ml portions of the hot solution to test tubes, suitably close or cover the tubes, sterilize at 121°, and allow the tubes to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of *Lactobacillus arabinosus (Lactobacillus plantarum)*, incubating for 16 to 24 hours at any selected temperature between 30° and 37° but held constant to within ± 0.5°, and finally store in a refrigerator. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

Culture medium : To each of a series of test-tubes containing 5.0 ml of *medium solution* add 5.0 ml of *water* containing 0.25 µg of calcium pantothenate. Plug the tubes, sterilise at 121°, and cool.

Preparation of inoculum : Transfer cells from the *stock culture of organism* to a sterile tube containing 10 ml of *culture medium*. Incubate for sixteen to twenty-four hours at a temperature between 30° and 37° but constant within ± 0.5.

Method

To similar test-tubes add, in duplicate, 1.0 and/or 1.5, 2.0, 3.0, 4.0 and 5.0 ml respectively, of the *standard solution*. To each tube and to 4 similar tubes containing no *standard solution* add 5.0 ml of *medium solution* and sufficient *water* to make 10 ml.

To similar test tubes add, in duplicate, volumes of the *test solution* corresponding to 3 or more of the levels listed above for the *standard solution*, including the levels of 2.0, 3.0, and 4.0 ml. To each tube add 5.0 ml of the *medium solution* and sufficient *water* to make 10 ml. Place one complete set of Standard and Assay tubes together in one tube rack and the duplicate set in a second rack or section of a rack, preferably in random order.

Cover the tubes of both series suitably to prevent contamination, and heat in an autoclave at 121° for 5 minutes. Cool, add 1 drop of inoculum to each tube, except 2 of the 4 tubes containing no *standard solution* (to serve as the uninoculated blanks), and mix. Incubate the tubes at a temperature between 30° and 37°, held constant to within ± 0.5° until, following 16 to 24 hours of incubation, there has been no substantial increase in turbidity in the tubes containing the highest level of standard during a 2-hour period.

Determine the transmittance * of the tubes in the following manner: Mix the contents of each tube, and transfer to an optical container if necessary. Place the container in a spectrophotometer that has been set at a specific wavelength between 540 nm and 660 nm, and read the transmittance when a steady state is reached. This steady state is observed a few seconds after agitation when the galvanometer reading remains constant for 30 seconds or more. Allow approximately the same time interval for the reading on each tube.

*Transmittance is the ratio of the intensity of the radiation passing through a layer of an absorbing substance to the intensity of the radiation passing into it.

With the transmittance set at 1.00 for the uninoculated blank, read the transmittance of the inoculated blank. With the transmittance set at 1.00 for the inoculated blank, read the transmittance for each of the remaining tubes. If there is evidence of contamination with a foreign micro-organism, disregard the result of the assay.

Calculation : Prepare a standard concentration-response curve as follows: For each level of the standard; calculate the response from the sum of the duplicate values of the transmittance as the difference $y = 2.00 - \Sigma$ (of transmittance). Plot this response on the ordinate of cross-section paper against the logarithm of the ml of *standard solution* per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, y, adding together the two transmittances for each level of the test solution. Read from the standard curve the logarithm of the volume of the *standard solution* corresponding to each of those values of y that fall within the range of the lowest and highest points plotted for the standard. Subtract from each logarithm so obtained the logarithm of the volume, in ml of the *test solution* to obtain the difference, x, for each dosage level. Average the values of x for each of three or more dosage levels to obtain $\bar{x} = M^1$, the log-relative potency of the *test solution*. Determine the quantity, in mg, of *calcium pantothenate R.S.* corresponding to the calcium pantothenate in the portion of material taken for assay as antilog M = antilog $(M^1 + \log R)$, in which R is the number of mg of calcium pantothenate that was assumed to be present in each mg (or capsule or tablet) of the material taken for assay.

4.3 TEST FOR COLONY-FORMING UNITS (CFU)

The number of colony-forming units (CFU) must be determined on the contents of at least five containers of the freeze-dried vaccine.

Special Medium

Asparagine	5.0 g
Potassium Dihydrogen Phosphate (KH_2PO_4)	5.0 g
Potassium Sulphate	0.5 g
Magnesium Citrate	1.5 g
Monosodium Glutamate	19.0 g
Glycerin	20.0 ml
Distilled Water	to 900.0 ml

100 ml of a 5 per cent w/v solution of bovine albumin that has been sterilised by filtration are added to the medium to produce a final concentration of 0.5 per cent of bovine albumin. The pH is adjusted to 7.0 with *5N sodium hydroxide*.

Method

Reconstitute each of five containers of the freeze-dried vaccine as for human use with the diluent stated on the label. From each container take a suitable volume of the vaccine and make appropriate dilutions with the *Special medium* up to a point where subsequent serial half-log dilutions will result in at least one tube containing between 10 and 50 CFU.

Make serial half-log dilutions in screw-capped test-tubes into which 4.5 ml aliquots of the *Special medium* have been dispersed. Add 2 ml of the thoroughly mixed vaccine to the first tube of the half-log series, mix thoroughly, transfer 2.0 ml from this tube to the next tube in the series. Repeat through each consecutive tube the process of mixing and serially transferring 2.0 ml and discard 2.0 ml from the last tube. After the serial half-log dilutions are completed, add quickly into each tube 0.5 ml of a 1.5 per cent w/v solution of *agar*, that has been cooled to 42°, to make a final concentration of 0.15 per cent agar and mix thoroughly the contents of the tubes. After mixing, incubate all the tubes at 35° to 37° for three to four weeks. From the test-tubes containing between 10 and 50 CFU and from the dilutions made, compute the number of colony-forming units (CFU) in the vaccine.

The vaccine passes the test if 0.1 ml of the reconstituted vaccine contains between 1×10^5 and 33×10^5 colony-forming units.

NOTE – The validity of the test for colony-forming units (CFU) must be determined by carrying out the test on the Standard Preparation of BCG Vaccine.

4.4. MICROBIOLOGICAL ASSAY OF CRYSTAL-VIOLET

Standard Preparation

Weigh accurately a suitable quantity of *crystal-violet R.S.* and dissolve in sufficient *water* to produce a solution containing 100 ug per ml of crystal-violet. Prepare a series of dilutions of this solution to produce five solutions (solu-

tions S_1 to S_5) containing in each ml 2 µg, 4 µg, 8 µg (median concentration), 16 µg and 32 µg of crystal-violet.

Test Organism

The test organism recommended for this assay is *staphylococcus aureus* (ATCC 6538P; NCTC 7447). Prepare the microbial suspension and the inoculated plates and determine the optimum inoculum as described under Test Organisms and Inoculum for Method A in Appendix 4.1, *microbiological assay of antibiotics*, Appendix 4.1.

Apparatus

Use rectangular glass trays or petri dishes (approx. 20 × 100 mm) with suitable covers. Use stainless steel or porcelain cylinders with the following dimensions, each dimension with a tolerance of ± 0.1 mm; outside diameter 8 mm; inside diameter 6 mm and length 10 mm. Instead of cylinders, holes 5 to 8 mm in diameter may be bored in the medium with a sterile borer.

Method

Using 21 ml of medium B (Table 3, Appendix 4.1,) 4 ml of seed layer of medium A(Table 3, Appendix 4.1) and the sample solution prepared as directed in the monograph, carry out Method A, Cylinder-plate or Cup-plate Method, one-level assay with standard curve described in Appendix 4.1.

Calculate the result of the assay by standard statistical methods. The fiducial limits of error are not less than 90 per cent and not more than 110 per cent of the estimated value.

4.5 MICROBIAL LIMIT TESTS

The following tests are designed for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The term 'growth' is used to designate the presence and presumed proliferation of viable micro-organisms.

Preliminary Testing

The methods given herein are invalid unless it is demonstrated that the test specimens to which they are applied do not, of themselves, inhibit the multiplication, under the test conditions, of micro-organisms that may be present. Therefore, prior to doing the tests, inoculate diluted specimens of the substance being examined, with separate viable cultures of *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is done by adding 1 ml of not less than 10^{-3} dilution of a 24-hour broth culture of the micro-organism to the first dilution (in *buffer solution pH 7.2*, Fluid Soybean-casein Digest Medium or Fluid Lactose Medium) of the test material and following the test procedure. If the organisms fail to grow in the relevant medium the procedure should be modified by (a) increasing the volume of diluent, the quantity of test material remaining the same or by (b) incorporating a sufficient quantity of a suitable inactivating agent in the diluents or by (c) combining the aforementioned modifications so as to permit growth of the media. If inhibitory substances are present in the sample 0.5 per cent of soy lecithin and 4 per cent of *polysorbate 20* may be added to the culture medium. Alternatively, repeat the test as described in the previous paragraph, using *fluid casein digest - soy lecithin - polysorbate 20 medium* to demonstrate neutralisation of preservatives or other antimicrobial agents in the test material.

Media

Culture media may be prepared as given below or dehydrated culture media may be used provided that, when reconstituted as directed by the manufacturer, they have similar ingredients and/or yield media comparable to those obtained from the formula given below:

Where agar is specified in a formula, use agar that has a moisture content of not more than 15 per cent. Where water is called for in a formula, use *purified water*. Unless otherwise indicated, the media should be sterilised by heating in an autoclave at 115° for thirty minutes.

Fluid soybean-casein digest medium : Prepare as directed under *test for sterility*, Appendix 4.6.

Soybean-casein digest agar medium

Pancreatic digest of casein	15.0 g
Papaic digest of soybean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 ml
pH after sterilisation:	7.3 ± 0.2

Fluid casein digest - soy lecithin - polysorbate 20 medium :

Pancreatic digest of casein	20 g
Soy lecithin	5 g
Polysorbate 20	40 ml
Water	960 ml

Dissolve the pancreatic digest of casein and soy lecithin in 960 ml of water, heating in a water bath at 48° to 50° for about 30 minutes to effect solution. Add 40 ml of polysorbate 20. Mix, and dispense as desired.

Bismuth sulphite agar

(1) Beef extract	6 g
Peptone	10 g
Agar	24 g
Ferric (III) citrate	0.4 g

Brilliant green	10 mg
Water	1000 ml

Dissolve with the aid of heat and sterilise by maintaining at 115° for thirty minutes.

(2) Ammonium bismuth citrate	3 g
Sodium sulphite	10 g
Anhydrous disodium hydrogen orthophosphate	5 g
D-Glucose monohydrate	5 g
Water	100 ml

Mix, heat to boiling, cool to room temperature, add 1 volume of solution (2) to 10 volumes of solution (1) previously melted and cooled to a temperature of 55°, and pour.

Bismuth sulphite agar should be stored at 2° to 8° for five days before use.

Brilliant green agar

Peptone	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Phenol red	0.08 g
Brilliant green	12.5 mg
Agar	12.0 g
Water	1000 ml

Mix, allow to stand for fifteen minutes, sterilise by maintaining at 115° for thirty minutes, and mix before pouring.

Cetrimide agar : Add 0.3 ml of a sterilised 10 per cent w/v solution of *cetrimide* to 100 ml of *nutrient agar.*

Cetrimide broth : Add 0.3 ml of a sterilised 10 per cent w/v solution of *cetrimide* to 100 ml of *nutrient broth.*

Deoxycholate citrate agar

Beef extract	5.0 g
Peptone	5.0 g
Lactose	10.0 g
Trisodium citrate	8.5 g
Sodium thiosulphate	5.4 g
Ferric (III) citrate	1.0 g
Sodium deoxycholate	5.0 g
Neutral red	0.02 g
Agar	12.0 g
Water	1000 ml

Mix and allow to stand for fifteen minutes. With continuous stirring, bring gently to the boil and maintain at boiling point until solution is complete. Cool to 80°, mix, pour and cool rapidly.

Care should be taken not to overheat Deoxycholate Citrate Agar during preparation. It should not be re-melted and the surface of the plates should be dried before use.

MacConkey's broth

Peptone	20 g
Sodium chloride	5 g
Sodium taurocholate	5 g
Water	1000 ml
Bromocresol purple solution	10 ml
Lactose	10 g

Dissolve the peptone, the sodium chloride, and the sodium taurocholate in the *water* with the aid of heat. Adjust to pH 8.0 and boil for twenty minutes. Cool, filter and adjust to pH 7.4. Add the lactose and the indicator solution, mix, and distribute in tubes containing inverted Durham tubes. Sterilise by maintaining at 115° for thirty minutes.

Mannitol-salt agar medium

Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	0.025 g
Water	1000 ml

Mix, then heat with frequent agitation, and boil for 1 minute to effect solution.

pH after sterilisation: 7.4 ± 0.2.

Nutrient agar : *Nutrient broth* gelled by the addition of 1.2 per cent of agar.

Nutrient broth

Beef extract	10 g
Peptone	10 g
Sodium chloride	5 g
Water	1000 ml

Dissolve with the aid of heat. Adjust to pH 8.0 to 8.4 with *5M sodium hydroxide* and boil for ten minutes. Filter, adjust to pH 7.2 to 7.4, and sterilise by maintaining at 115° for thirty minutes.

Peptone water

Peptone	10 g
Sodium chloride	5 g
Water	1000 ml

Dissolve with the aid of heat, adjust to pH 8.0 to 8 4, and boil for twenty minutes. Filter, adjust to pH 7.2 to 7.4, and sterilise by maintaining at 115° for thirty minutes.

Selenite F broth

Peptone	5 g
Lactose	4 g
Disodium hydrogen orthophosphate	10 g
Sodium hydrogen selenite	4 g
Water	1000 ml

Dissolve, distribute in sterile containers and sterilise by maintaining at 100° for thirty minutes.

Tetrathionate broth

Beef extract	0.9 g
Peptone	4.5 g
Yeast extract	1.8 g
Sodium chloride	4.5 g
Calcium carbonate	25.0 g
Sodium thiosulphate	40.7 g
Water	1000 ml

Mix, bring to the boil, cool to below 45°, and add a solution of 6 g of iodine and 5 g of *potassium iodide* in 20 ml of *water*. Mix, and distribute in sterile containers.

Triple sugar iron agar

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Lactose	10.0 g
Sucrose	10.0 g
D-Glucose monohydrate	1.0 g
Iron (II) sulphate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Phenol red	24 mg
Agar	13.0 g
Water	1000 ml

Mix, allow to stand for fifteen minutes, bring to and maintain at boiling point until solution is complete, mix, distribute in tubes and sterilise by maintaining at 115° for thirty minutes. Allow to set in a sloped form with a butt about 2.5 cm long.

Urea broth

Potassium dihydrogen orthophosphate	9.1 g
Anhydrous disodium hydrogen orthophosphate	9.5 g
Urea	20.0 g
Yeast extract	0.1 g
Phenol red	0.01 g
Water	1000 ml

Mix, sterilise by filtration and distribute aseptically in sterile containers.

Vogel-Johnson agar medium

Pancreatic digest of casein	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dibasic potassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Agar	16.0 g
Phenol red	25.0 mg
Water	1000 ml

Boil the solution of solids for 1 minute. Sterilize, cool to between 45° and 50° and add 20 ml of sterile potassium tellurite solution (in 1 in 100)

pH after sterilisation: 7.2 ± 0.2.

Sampling : Use separate 10 ml or 10 g specimens for each of the tests specified in the individual monograph.

Methods

(1) Total microbial count : Use the Plate method for substances that are sufficiently soluble or translucent to permit the use of that method; otherwise, use the Multiple-tube method.

Dissolve or suspend 10 g of the substance being examined in sufficient *buffer solution, pH 7.2, Fluid Soybean-Casein Digest Medium*, or *Fluid Casein Digest-Soylecithin-polysorbate 20 Medium* to produce 100 ml. Perform the test for absence of inhibitory (anti-microbial) properties as described under *Preliminary Testing* before the determination of Total Microbial Count. Add the substance being examined, to the medium not more than one hour after preparing the appropriate dilutions for inoculation.

(a) Plate method – Dilute further, if necessary, the sample liquid prepared as described above, so that 1 ml will be expected to yield between 30 and 300 colonies. Pipette 1 ml of the final dilution into each of two sterile petri dishes. Immediately add to each dish 15 to 20 ml of *Soybean-casein digest agar medium* that has previously been melted and cooled to about 45°. Cover the petri dishes, mix the sample with the agar by tilting or rotating the dishes, and allow the contents to solidify at room temperature. Invert the petri dishes, and incubate for forty-eight to seventy-two hours. After incubation examine the plates for growth, count the number of colonies, and express the average for the two plates in terms of number of micro-organisms per g of the substance. If no colonies are recovered from the dishes representing the initial 1:10 dilution of the substance, express the results as "less than 10 micro-organisms per g of substance".

(b) Multiple-tube method – Into each of fourteen test tubes of similar size place 9.0 ml of sterile *Fluid-soybean-casein digest medium*. Arrange twelve of the tubes in four sets of three tubes each. Put aside one set of three tubes to serve as the controls. Into each of three tubes of one set ("100") and into a fourth tube (A) pipette 1 ml of the solution of suspension of the specimen, and mix. From tube A pipette 1 ml of its contents into the one remaining tube (B) not included in a set, and mix. These two tubes contain 100 mg (or 100 µl) and 10 mg (or 10 µl) of the specimen, respectively. Into each of the second set ("10") of three tubes pipette 1 ml from tube A, and into each tube of the third set ("1") pipette 1 ml from tube B. Discard the unused contents of tubes A and B. Close well, and incubate all of the tubes. Following the incubation period, examine the tubes for growth; the three control tubes remain clear and the observations in the tubes containing the specimen, when interpreted by reference to Table 1, indicate the most probable number of micro-organisms per g or per ml of specimen.

TABLE 1 – Most Probable Total Count by Multiple-tube Method

Observed combination of numbers of tubes showing growth in each set			Most probable number of micro-organisms per g or per ml
No. of mg (or ml) of specimen per tube			
100 (100 µl)	10 (10 µl)	1 (1 µl)	
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

(2) Escherichia coli : Place the prescribed quantity in a sterile screw-capped container, add 50 ml of *nutrient broth*, shake, allow to stand for one hour (four hours for gelatin) and shake again. Loosen the cap and incubate at 37° for eighteen to twenty-four hours.

Primary test – Add 1.0 ml of the enrichment culture to a tube containing 5 ml of *MacConkey's broth*. Incubate in a water-bath at 36° to 38° for forty-eight hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test – Add 0.1 ml of the contents of the tube showing acid and gas to each of two tubes containing (a) 5 ml of *MacConkey's broth*, and (b) 5 ml of *peptone water*. Incubate in a water-bath at 43.5° to 44.5° for twenty-four hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole add 0.5 ml of *Kovac's reagent*, shake well, and allow to stand for one minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of *Escherichia coli*.

Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Escherichia coli* (NCTC 9002), prepared from a twenty-four hour culture in *nutrient broth*, to 5 ml of *MacConkey's broth*. The test is invalid if the results do not indicate that the control contains *Escherichia coli*.

(3) Salmonellae : Place the prescribed quantity in a sterile screw-capped jar, add 100 ml (unless otherwise directed) of *nutrient broth*, shake, allow to stand for one hour (four hours for gelatin), and shake again. Loosen the cap and incubate at 37° for eighteen to twenty-four hours.

Primary test – Add 1.0 ml of the enrichment culture to each of two tubes containing (a) 10 ml of *salenite F broth*, and (b) 10 ml of *tetrathionate broth* and incubate at 36° to 38° for forty-eight hours. From each of these two cultures inoculate three plates containing a layer of

TABLE 1

Medium	Description of Colony
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate citrate agar	Colourless and opaque, with or without black centres
Bismuth sulphite agar	Black or green

(i) *brilliant green agar*, (ii) *desoxycholate citrate agar*, and (iii) *bismuth sulphite agar* Incubate the plates at 36° to 38° for eighteen to twenty-four hours. If any colonies conforming to the description in Table 1 are produced, carry out the secondary test.

Secondary test – Subculture any colonies showing the characteristics given in Table 1 in *triple sugar iron agar*, by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle and at the same time inoculate a tube of *urea broth*. Incubate at 36° to 38° for eighteen to twenty-four hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with absence of a red colour in the urea broth, indicates the presence of salmonellae. If acid but no gas is produced in the stab culture, the identity of the organisms should be confirmed by agglutination tests.

Carry out a control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Salmonellae abony* (NCTC 6017), prepared from a twenty-four hour culture in *nutrient broth*, for the inoculation of tubes (a) and (b). The test is invalid if the results do not indicate that the control contains Salmonellae.

(4) Pseudomonads : Place the prescribed quantity in a sterile screw-capped jar containing 100 ml of *cetrimide broth* and incubate at 30° to 32° for seventy-two hours. Subculture on a plate containing a layer of *cetrimide agar* and incubate at 30° to 32° for forty-eight hours. Examine the resulting growth by Gram's stain and apply the oxidase test; Gram-negative bacilli giving a positive oxidase test indicate the presence of pseudomonads.

Carry out a control test by repeating the test adding the prescribed quantity and a volume of broth containing 10 to 50 *Pseudomonas aeruginosa* (NCTC 6750), prepared from a twenty-four hour culture in *nutrient broth*, to a sterile, screw-capped jar containing 100 ml of *cetrimide broth*. The test is invalid if the results do not indicate that the control contains pseudomonads.

Oxidase test – Place 2 or 3 drops of a freshly prepared 1 per cent w/v solution of *NNN'N'-tetramethyl-p-phenylenediammonium dichloride* on a piece of filter paper (Whatman No. 1 is suitable) and smear with the suspect colony; if a purple colour is produced within five to ten seconds, the test is positive.

(5) Staphylococcus aureus : Place the prescribed quantity in a sterile screw-capped jar containing 100 ml of *fluid soybean-casein digest medium* and incubate at 30° to 32° for seventy-two hours. Subculture on a plate containing a layer of *mannitol salt agar medium* or *Vogel-Johnson agar medium* and incubate at 30° to 32° for forty-eight hours. Examine the resulting growth by Gram's stain and apply the coagulase test. Gram-positive cocci (in clusters) in yellow colonies (in mannitol salt agar medium) and in colonies, black surrounded by yellow zones (in Vogel-Jhonson agar medium), and giving a positive coagulase test indicate the presence of *Staphylococcus aureus*.

Carry out a control test by repeating the test, adding the prescribed quantity and a volume of broth containing 10 to 50 *Staphylococcus aureus* (ATCC 6538; NCTC 10788), prepared from a twenty-four hour culture in *fluid soybean-casein digest medium*, to a sterile screw-capped jar containing 100 ml of *fluid soybean-casein digest medium*. The test is invalid if the results do not indicate that the control contains *Staphylococcus aureus*.

Coagulase test – Transfer representative suspect colonies from the agar surfaces of the mannitol salt agar medium or Vogel-Johnson agar medium to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a water-bath at 37°, examining the tubes at three hours and subsequently at suitable intervals upto twenty-four hours. If coagulation in any degree is observed, the test is positive.

4.6 TESTS FOR STERILITY

The tests for sterility are intended for detecting the presence of viable forms of bacteria, fungi and yeasts in or on pharmacopoeial preparations. The tests must be carried out under conditions designed to avoid accidental contamination of the product during the test. Precautions taken for this purpose should not adversely affect any micro-organisms which should be revealed in the test.

The working conditions in which the tests are performed should be monitored regularly by sampling the air and surfaces of the working area and by carrying out control tests. The tests are based upon the principle that if bacteria or fungi are placed in a medium which provides nutritive material and water, and kept at a favourable temperature, the organisms will grow and their presence can be indicated by a turbidity in the originally clear medium.

The probability of detecting viable micro-organisms in

TABLE 1

Number of Items in the Batch	Minimum Number of Items Recommended to be Tested
1. *Injectable preparations*	
Not more than 100 containers	10 per cent or 4 containers whichever is the greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2 per cent or 20 containers whichever is the less
2. *Ophthalmic and other non-injectable preparations*	
Not more than 200 containers	5 per cent or 2 containers whichever is the greater
More than 200 containers	10 containers
3. *Surgical dressings*	
Not more than 100 packages	10 per cent or 4 packages whichever is the greater
More than 100 but not more than 500 packages	10 packages
More than 500 packages	2 per cent or 20 packages whichever is the less
4. *Bulk solids*	
Less than 4 containers	Each container
4 containers but not more than 50 containers	20 per cent or 4 containers whichever is the greater
More than 50 containers	2 per cent or 10 containers whichever is the greater

If the product is presented in the form of single-dose containers, apply the scheme shown above for Injectable Preparations.

the *tests for sterility* increases with the number present in a given amount of the preparation being tested and varies according to the species of micro-organisms present. Very low levels of contamination cannot be detected on the basis of random sampling of a batch (A batch may be defined for the purposes of these tests as a homogeneous collection of sealed containers prepared in such a manner that the risk of contamination is the same for each of the units in it). Moreover, if contamination is not uniform throughout the batch random sampling cannot detect contamination with certainty. Compliance with the *tests for sterility* alone cannot therefore constitute absolute assurance of freedom from microbial contamination. Greater assurance of sterility must come from reliable manufacturing procedures.

The *test for sterility* are designed to reveal the presence of micro-organisms in the samples used in the tests; interpretation of results is based on the assumption that the contents of every container in the batch, had they been tested, would also have complied with the tests. Since every container cannot be tested, a sufficient number of containers should be examined to give a suitable degree of confidence in the results of the tests.

No sampling plan for applying the tests to a specified proportion of discrete units selected from a batch is capable of demonstrating that all of the untested units are in fact sterile. Therefore in determining the number of units to be tested, the manufacturer should have regard to the environmental conditions of manufacture, the volume of preparation per container and other special considerations particular to the preparation being examined. Table 1 gives guidance on the minimum number of items recommended to be tested in relation to the number of items in the batch on the assumption that the preparation has been manufactured under conditions designed to exclude contamination.

Culture Media

Media for the tests may be prepared as described below, or dehydrated mixtures yielding similar formulations may be used provided that when reconstituted as directed by the manufacturer, they have growth-promoting properties equal to or superior to those from the formulae given herein. Other media may be used, provided that they have been shown to sustain the growth of a wide range of micro-organisms.

Medium for aerobic and anaerobic bacteria : Two types of media are recommended; fluid thioglycollate medium for use with clear fluid products and the alternative thioglycollate medium for the testing of turbid and viscid products.

1. Fluid thioglycollate medium — For use in testing of clear, fluid products.

L-Cystine	0.5 g
Sodium chloride	2.5 g
Dextrose ($C_6H_{12}O_6.H_2O$)	5.5 g
Granular agar (less than 15 per cent moisture by weight)	0.75 g
Yeast extract (water-soluble)	5.0 g

Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycollic acid	0.5 g 0.3 ml
Resazurin (1.10% fresh solution)	1.0 ml
Distilled water	1000 ml
Final pH after sterilisation	7.1 ± 0.2

Mix the ingredients other than the thioglycollate and the resazurin, in the order given above in a mortar, with thorough grinding. Stir in some heated water, transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water-bath. Add the sodium thioglycollate, then *1N sodium hydroxide* so that the pH of the completed and sterilized medium will be 7.1 ± 0.2. Reheat the solution, but do not boil, filter (if necessary) through a moistened filter paper and add the resazurin solution. Distribute into suitable vessels and sterilize by autoclaving for 18 - 20 minutes at 121° (15 lbs psi, approximately 1.0 kg/cm^2). Cool promptly to 25° and store at 20° - 30°, avoiding excessive light. If more than 30 per cent of the uppermost portion of the medium has changed to a pinkish colour it is unsuitable for use. Medium more than three weeks old should not be used.

Use Fluid thioglycollate medium by incubating it under aerobic conditions.

II. Alternative thioglycollate medium – For use in testing the sterility of turbid and viscid products instead of fluid thioglycollate, provided it is freshly prepared or is boiled and cooled just prior to use.

L-Cystine	0.5 g
Sodium chloride	2.5 g
Dextrose ($C_6H_{12}O_6H_2O$)	5.5 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycollic acid	0.5 g 0.3 ml
Distilled water	1000 ml
Final pH after sterilisation	7.1 ± 0.2

Heat the ingredients in a suitable container until solution is affected. Mix and adjust the reaction with *1N sodium hydroxide*, if necessary, so that after sterilisation, the medium will have a pH of 7.1 ± 0.2. Filter if necessary, place in suitable vessels, and sterilize in an autoclave for 18-20 minutes at 121° (15 lbs psi, approximately 1.0 kg/cm^2).

The medium is freshly prepared or heated in a steam-bath and allowed to cool just prior to use. Do not reheat.

Use Alternative thioglycollate medium in a manner that will assure anaerobic conditions for the duration of the incubation period.

Medium for fungi and aerobic bacteria

Soybean-casein digest medium

Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Dextrose ($C_6H_{12}O_6H_2O$)	2.5 g
Distilled water	1000 ml
Final pH after sterilisation	7.3 ± 0.2

Dissolve the solids in the *water*, warming slightly to effect solution. Cool to room temperature and add, if necessary, sufficient *0.1N sodium hydroxide* to give a final pH after sterilisation of between 7.1 and 7.5. Filter, if necessary, to clarify, distribute into suitable containers and sterilise in an autoclave at 121° for about twenty minutes.

Use soybean-casein digest medium by incubating it under aerobic conditions.

The media used should comply with the following tests carried out before or in parallel with the test on the preparation being examined.

1. **STERILITY** – Incubate portions of the media intended for the detection of bacteria at 30° to 35° and portions of those intended for the detection of fungi at 20° to 25° for not less than seven days. No growth of micro-organisms occurs.

2. **NUTRITIVE PROPERTIES** – Inoculate each medium to be used in the test with about 100 viable micro-organisms of the following types as appropriate; an aerobe such as *Staphylococcus aureus* (NCTC* 7447), a spore-forming aerobe such as *Bacillus subtilis* (ATCC** 6633; NCTB*** 8054), an anaerobe such as *Clostridium sporogenes* (ATCC 11437; NCTC 532) and a fungus such as *Candida albicans* (ATCC 10231; NCYC† 854); each type should be incubated in a separate portion of the medium. Incubate the inoculated media at the appropriate temperature as specified under Sterility for not more than seven days. Early and copious growth of the micro-organisms occurs.

3. **EFFECTIVENESS OF MEDIA** – (a) Prepare at least two containers of the medium to be used for testing for anaerobic bacteria and to each add the preparation being examined in the same quantity, and treated in the same manner, as in

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* National Collection of Type Cultures, Central Public Health Laboratory, Colindale Annexure, London WC1E 7HT, England.

** American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md 20852.

*** National Collection of Industrial Bacteria, Tory Research Station, P.O. Box 31, Abbey Road, Aberdeen AB9 8DG Scotland.

† National Collection of Yeast Cultures, The Brewing Research Foundation, Lyttel Hall, Nutfield; Redhill RH, GHY England.

the *tests for sterility*. Inoculate the containers with about 100 viable spores (0.1 ml of a suitably diluted suspension) of a suitable strain of an anaerobic organism such as *Clostridium sporogenes*. Prepare a similar set of containers omitting the preparation being examined and inoculate them in the same manner. Incubate all the containers at 30° to 35° for not more than seven days.

(b) Prepare at least two containers of the medium to be used for testing for aerobic bacteria and to each add the preparation being examined in the same quantity, and treated in the same manner, as in the *tests for sterility*. Inoculate the containers with about 100 viable organisms (0.1 ml of a suitably diluted suspension) of a suitable strain of an aerobic organism such as *Staphylococcus aureus*. Prepare a similar set of containers omitting the preparation being examined and inoculate them in the same manner. Incubate all the containers at 30° to 35° for not more than seven days.

(c) Prepare at least two containers of the culture medium to be used for testing for fungi and to each add the preparation being examined in the same quantity, and treated in the same manner, as in the *test for sterility*. Inoculate the containers with about 100 viable organisms (0.1 ml of a suitably diluted suspension) of a suitable strain of a fungus such as *Candida albicans*. Prepare two similar containers omitting the preparation being examined and inoculate them in the same manner. Incubate all the containers at 20° for not more than seven days.

If during the incubation the microbial growth is similar (early and copious) in the presence and the absence of the preparation being examined, the latter has no antimicrobial action under the conditions of the test and the *test for sterility* may be carried out without modification. If the cultures containing the preparation being examined show weaker growth, delayed growth or no growth of micro-organisms when compared with the controls, the preparation being examined has an antimicrobial activity which must be eliminated by filtration, dilution or neutralisation before or during the Tests for Sterility. Establish that the antimicrobial properties of the preparation have been effectively removed by repeating the test for Effectiveness of media.

Tests for Sterility

The *tests for sterility* comprise the test for aerobic and anaerobic bacteria and the test for fungi.

The tests may be carried out using Method A, Membrance Filtration or Method B, Direct Inoculation. Method A is to be preferred where the substance being examined is (a) an oil, (b) an ointment that can be put into solution, (c) a non-bacteriostatic solid not readily soluble in culture medium, and (d) a soluble powder or a liquid that possesses inherent bacteriostatic and fungistatic properties.

For liquid products where the volume in a container is 100 ml or more, only Method A should be employed.

General procedure : Cleanse the exterior surface of ampoules and closures of vials and bottles with a suitable antimicrobial agent and gain access to the contents in a suitable aseptic manner. If the contents are packed in a container under vacuum, admit sterile air by means of a suitable sterile device, such as a needle attached to a syringe barrel filled with non-absorbent cotton.

Select the number of samples to be tested from Table 1 and use them for the culture medium for bacteria and the culture medium for fungi. Unless otherwise directed in the individual monograph or in any other section of this monograph, incubate the test mixture for fourteen days with *fluid thioglycollate medium* or *alternative thioglycollate medium*, wherever so indicated, at 30° to 35° and with *soybean-casein digest medium* at 20° to 25°.

Method A: Membrane Filtration

The method needs exceptional skill and special knowledge; it also calls for the routine use of positive and negative controls. A suitable positive control is the occasional use of known contaminated solution containing a few (approximately 10 microbial cells in the total volumes employed) micro-organisms of differing types.

(a) Apparatus

A suitable unit consists of a closed reservoir and a receptacle between which a properly supported membrane of appropriate porosity is placed. A membrane generally suitable for sterility testing has nominal porosity of 0.45 μ, a diameter of approximately 47 mm, and a flow-rate of 55 to 75 ml of water per minute at a pressure of 70 cm of mercury. Preferably assemble and sterilize the entire unit with the membrane in place, prior to use. Where the sample to be tested is an oil, sterilize the membrane separately, and after thorough drying, assemble the unit, using aseptic precautions.

(b) Diluting fluids

Fluid A — Dissolve 1 gram of peptic digest of animal tissue (such as bacteriological peptone) or the equivalent in *water* to make one litre, filter or centrifuge to clarify, adjust to pH 7.1 ± 0.2, dispense into flasks in 100 ml quantities, and sterilize at 121° for 18-20 minutes.

Fluid B — If the test sample contains lecithin or oil, use Fluid A to each litre of which has been added 1 ml of *polysorbate 80*, adjust to pH 7.1 ± 0.2, dispense into flasks and sterilize at 121° for 18 - 20 minutes.

NOTE — Any diluent that does not manifest antimicrobial activity and does not effect the porosity of the membrane may be suitable for dissolving a preparation under test for sterility.

(c) Quantities of sample to be used

(i) For Injectable Preparations — Whenever possible use the whole contents of a container, but in any case not

less than the quantities prescribed in Table 2, diluting where necessary to about 100 ml with a suitable sterile diluent, such as *Fluid A*.

(ii) For Ophthalmic and other Non-injectable Preparations – Take an amount within the range prescribed in column A of Table 3, if necessary, using the contents of more than one container, and mix thoroughly. For each medium use the amount specified in column B of Table 2, taken from the mixed sample.

(d) Method of test

(i) Aqueous Solutions – Prepare each membrane by aseptically transferring a small quantity (sufficient to moisten the membrane) of Fluid A on to the membrane and filtering it. For each medium to be used, transfer aseptically into two separate membrane filter funnels or to separate sterile pooling vessels prior to transfer not less than the quantity of the preparation being examined that is prescribed in Table 2 or 3. Alternatively, transfer aseptically the combined quantities of the preparation being examined prescribed for the two media into one membrane. Draw the liquid rapidly through the filter with the aid of vacuum. If the solution being tested has antimicrobial properties, wash the membrane(s) by filtering through it (them) not less than three successive quantities, each of approximately 100 ml, of sterile *Fluid A*. The number of quantities of fluid used should be sufficient to allow growth of a small inoculum of organisms (approximately 50) sensitive to the antimicrobial substance in the presence of the residual inhibitory material on the membrane.

After filtration, aseptically remove the membrane(s) from the holder, cut the membrane in half, if only one is used, immerse the membrane, or one-half of the membrane, in 100 ml of *soybean-casein digest medium*, and incubate at 20° to 25° for not less than seven days. Similarly, immerse the other membrane, or other half of the membrane, in 100 ml of *fluid thioglycollate medium*, and incubate at 30° to 35° for not less than 7 days.

(ii) Liquids Immiscible with Aqueous Vehicles and Suspensions – Carry out the test described under *aqueous solutions* but add a sufficient quantity of *Fluid A* to the pooled sample to achieve rapid filtration. Sterile enzyme preparations such as penicillinase or cellulase may be added to *Fluid A* to aid in dissolving insoluble substances. If the substance under test contains lecithin, use *Fluid B* for diluting.

(iii) Oils and Oily Solutions – Filter oils or oily solutions of sufficiently low viscosity without dilution through a dry membrane. Dilute viscous oils as necessary with a suitable sterile diluent such as *isopropyl myristate*, that has been shown not to have antimicrobial properties under the conditions of the test. Allow the oil to penetrate the membrane and filter, applying pressure or suction gradually. Wash the membrane by filtering through it at least three successive quantities of approximately 100 ml.

TABLE 2

Quantity in each Container	Minimum Quantity to be used for each Culture Medium
For liquids	
Less than 1 ml	The whole contents of a container
1 ml or more but less than 4 ml	Half of the contents of a container
4 ml or more but less than 20 ml	2 ml
20 ml or more but less than 100 ml	10 per cent of the contents of a container unless otherwise indicated in the monograph
100 ml or more	Not less than half the contents of the container unless otherwise specified.
For solids	
Less than 50 mg	The whole contents of a container
50 mg or more but less than 200 mg	Half the contents of a container
200 mg or more	100 mg

TABLE 3

Type of Preparation	Quantity to be Mixed (A)	Quantity to be used for each Culture Medium (B)
Ophthalmic solutions and other non-injectable liquid preparations	10 to 100 ml	5 to 10 ml
Other preparations; preparations soluble in water or appropriate solvents; insoluble preparations to be suspended or emulsified (ointments and creams)	1 to 10 g	0.5 to 1 g

of sterile *Fluid B* or any other suitable sterile diluent. Complete the test described under *Aqueous Solutions* beginning at the words "After filtration. . . . .".

(iv) Ointments and Creams – Dilute ointments in a fatty base and emulsions of the water-in-oil type, to give a fluid concentration of 1 per cent w/v, by heating, if necessary, to not more than 40° with a suitable sterile diluent that does not have antimicrobial properties under the conditions of the test, such as *isopropyl myristate*, previously rendered sterile by filtration through a 0.22 µm membrane filter. Filter as rapidly as possible and complete the test as described under *Oils and Oily Solutions* beginning at the words "wash the membrane by....". In exceptional cases it may be necessary to heat the substance to not more than 45° and to use warm solutions for washing the membrane.

NOTE – For ointments and oils that are insoluble is isopropyl myristate, use Method B.

(v) Soluble Solids – For each medium, dissolve not less than the quantity of the preparation being examined, that is prescribed in Tables 2 and 3, in a suitable sterile solvent such as *Fluid A* and carry out the test described under *Aqueous Solutions* using a membrane or membranes appropriate to the chosen solvents.

(vi) Sterile Devices – Aseptically pass a sufficient volume of *Fluid B* through each of not less than twenty devices so that not less than 100 ml is recovered from each device. Collect the fluids in sterile containers, and filter the entire volume collected through membrane filter funnel(s) as described under *Aqueous Solutions*.

Method B : Direct Inoculation

(a) Quantities of sample to be used

The quantity of the substance or preparation being examined which is to be used for inoculation in the culture media varies according to the quantity in each container and is given in Table 4 along with the volume of medium to be used.

(b) Method of test

(i) Aqueous Solutions and Suspensions – The tests for bacterial and fungal contamination are carried out on the same sample of the preparation being examined. When the quantity in a single container is insufficient to carry out the tests, the combined contents of two or more containers are used to inoculate the different media.

Remove the liquid from test containers with a sterile pipette or with a sterile syringe and needle. Aseptically transfer the specified volume of the material from each containers to a vessel of culture medium. Mix the liquid with the medium, but do not aerate excessively. Incubate in the specified media for not less than fourteen days.

When the material being tested renders the medium turbid, so that the presence or absence of microbial growth cannot be determined readily by visual examination, transfer suitable portions of the medium to fresh vessels of the same medium between the third and seventh days after the test is started. Continue incubation of the original and of the transfer vessels for not less than seven additional days after the transfer and for a total of not less than fourteen days.

(ii) Oils and Oily Solutions – Use media to which have been added 0.1 per cent w/v of (4-tert-octylphenoxy)polyethoxyethanol, 1 per cent w/v of *polysorbate 80* or other suitable emulsifying agents, in an appropriate concentration, shown not to have any antimicrobial properties under the conditions of the test. Carry out the test as described under *Aqueous Solutions and Suspensions*.

Cultures containing oily preparations should be shaken gently each day. However, when Fluid Thioglycollate Medium or Alternative Thioglycollate Medium is used for the detection of anaerobic micro-organisms, shaking or mixing should be kept to a minimum to maintain anaerobic conditions.

(iii) Ointments – Prepare by diluting ten-fold in a suitable sterile diluent such as *Fluid B* or any other aqueous vehicle capable of dispersing the test material homogeneously throughout the fluid mixture (Before use, test the

TABLE 4

Container Content	Minimum Quantity Product	Minimum Volume of Culture Medium (ml)
For liquids		
Less than 1 ml	Total contents	15
1 ml or more but less than 5 ml	Half the contents	20
5 ml or more but less than 20 ml	2 ml	20
20 ml or more but less than 50 ml	5 ml	40
50 ml or more but less than 100 ml	10 ml	80
For solids		
Less than 50 mg	Total contents	40
50 mg or more but less than 200 mg	Half the contents	80
200 mg or more	100 mg	80

dispersing agent to ascertain that in the concentration used it has no significant antimicrobial effects during the time interval for all transfers). Mix 10 ml of the fluid mixture so obtained with 80 ml of medium and proceed as directed under *Aqueous Solutions and Suspensions*.

(iv) Solids – Transfer the quantity of the preparation to be examined to the quantity of medium specified in Table 4 and mix, the conditions of incubation being the same as for *Aqueous Solutions and Suspensions*. Proceed as directed under *Aqueous Solutions and Suspensions* beginning at the words "when the material. . . .".

(v) Sterile Devices—For articles of such size and shape as to permit complete immersion in not more than 1000 ml of culture medium, test the intact article, using the appropriate media, and incubate as directed under **General procedure**

For transfusion or infusion assemblies or where the size of an item makes immersion impracticable and only the liquid pathway must be sterile, flush the lumen of each of twenty units with a sufficient quantity of *fluid thioglycollate medium* and the lumen of each of twenty units with a sufficient quantity of *soybean-casein digest medium* to yield a recovery of not less than 15 ml of each medium, and incubate with not less than 100 ml of each of the two media as directed under **General procedure**. For devices in which the lumen is so small that Fluid Thioglycollate Medium will not pass through, substitute Alternative *Thioglycollate medium* for *Fluid Thioglycollate Medium* and incubate that inoculated medium anaerobically.

Where the presence of the specimen being tested, in the medium interferes with the test because of bacteriostatic or fungistatic action, rinse the article thoroughly with a minimal amount of *Fluid A*. Recover the rinse fluid, and test as described for *Sterile Devices* under Method A.

Observation and Interpretation of Results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If no evidence of growth is found, the preparation being examined passes the *tests for sterility*. If evidence of microbial growth is found, reserve the containers showing this and, unless it can be demonstrated by any other means that their presence is due to causes unrelated to the preparation being examined and hence that the *tests for sterility* is invalid and may therefore be recommended, perform a re-test using the same number of samples, volumes to be tested and the media as in the original test. If no evidence of microbial growth is then found, the preparation being examined passes the *tests for sterility*. If evidence of microbial growth is found, isolate and identify the organism. If they are not readily distinguishable from those growing in the containers reserved in the first test the preparation being examined fails the *tests for sterility*. If they are readily distinguishable from those growing in the containers reserved in the first test, perform a second re-test using twice the number of samples. If no evidence of microbial growth is found in the second re-test, the preparation being examined passes the *tests for sterility*. If evidence of growth of any microorganisms is found in the second re-test the preparation being examined fails the *tests for sterility*.

4.7 DETERMINATION OF THIOMERSAL

Standard Preparation

Weigh accurately about 0.1 g of *thiomersal*, previously dried "in vacuo" over *phosphorous pentoxide* for twenty-four hours and dissolve in sufficient *water* to produce 1000.0 ml. Prepare just before use, dilutions of this solution with *water* to produce standard solutions containing in each ml 5.0 µg (low level) and 10.0 µg (high level) of thiomersal.

Test Medium

Pancreatic digest of casein	10.0 g
Beef extract	3.0 g
Yeast extract	1.5 g
Agar	15.0 g
Sodium chloride	3.0 g
Sucrose	1.0 g
Water	to 1000 ml

Dissolve with the aid of heat, adjust the pH to between 7.4 and 7.6 and sterilise by maintaining at 121° for twenty minutes.

Test Organism

The test organism recommended for this assay is *Micrococcus flavus* (ATCC 10240; NCIB 8994).

Preparation of Inoculum and Assay Plates

Maintain the test organism by regular sub-culturing on slopes of the *test medium*. After incubation at 30° for eighteen to twenty-four hours, emulsify the growth in about 10 ml of sterile *normal saline*.

To 150 ml of the *test medium* kept at a temperature between 46° and 48° add 1.5 ml of the well-shaken inoculum, swirl gently and mix well. Immediately pour the inoculated medium into petri-dishes or large rectangular plates to give a depth of 3 to 4 mm. Cool and transfer to a cold place until the medium is set. Prior to use, prepare cavities approximately 8 mm in diameter in the solid medium.

Method

From an accurately measured volume of the preparation

being examined prepare sample solutions containing two levels, namely about 5 μg and 10 μg of thiomersal and carry out Method A, Cylinder-plate or Cup-plate Method, two-level factorial assay described in Appendix 4.1, *microbiological assay of antibiotics*; incubate the plates at 30° for about eighteen hours.

Calculate the result by standard statistical methods. The fiducial limits of error are not less than 90 per cent and not more than 110 per cent of the estimated value.

If the value obtained for the samples is lower than 50 per cent or greater than 150 per cent of the standard, the determination is invalid and should be repeated using higher or lower dilutions of the sample solution.

5. Physical Tests and Determinations

5.1 TESTS FOR ABSORBENCY

Apparatus

A dry, cylindrical copper wire basket, 8.0 cm high and 5.0 cm in diameter, fabricated from wire of diameter 0.4 mm, and having a mesh aperture of 1.5 to 2.0 cm; the basket weighs 2.4 to 3.0 g.

1. Test for Sinking Time

Weigh the basket to the nearest centigram. Take five portions, each of approximately 1 g, from different places in the sample, pack loosely in the basket and weigh the packed basket to the nearest centigram. Hold the basket with its long axis in the horizontal position and drop it from a height of about 10 mm into *water* at 20° contained in a beaker at least 12 cm in diameter and filled to a depth of 10 cm. Measure with a stopwatch the time taken by the basket to sink below the surface of the water. Repeat the procedure on two further samples and calculate the average value.

Absorbent cotton wool requires not more than ten seconds.

2. Test for Water-holding Capacity

After the sinking time has been recorded in Test 1, remove the basket from the water, allow it to drain for thirty seconds with its long axis in the horizontal position, transfer it to a tared beaker, and weigh to the nearest centigram. Calculate the weight of water retained by the sample. Repeat the procedure on two further samples and calculate the average value.

Absorbent cotton wool retains not less than 23.0 g of water per g.

5.2 DETERMINATION OF ALCOHOL

Unless otherwise specified in the individual monograph, distillation methods are to be used for the determination of alcohol.

A. Distillation Methods

The following general methods are to be used for the determination of alcohol, unless otherwise specified in the individual monograph. When carrying out the methods, precaution should be taken to minimise the loss of alcohol by evaporation.

Apparatus

The apparatus (*see* Fig. 1) consists of a round-bottomed 500-ml flask (A) fitted with a distillation head (B) with a steam trap and attached to a vertical condenser (C). The latter is fitted at its lower part with a tapered tube (D) which carries the distillate into a 100-ml or 200-ml volumetric flask. The volumetric flask is immersed in a water-ice mixture (E) during the distillation. A disc having a circular aperture 6 cm in diameter is placed under the flask (A) to reduce the risk of charring of any dissolved substances.

Method I

Transfer 25 ml of the sample, accurately measured at 25°, to the distillation flask. Dilute with 150 ml of *water* and add a little pumice powder. Attach the distillation head and condenser. Distil and collect not less than 90 ml of distillate into a 100-ml volumetric flask. Adjust the temperature to 25° and dilute with *water* at 25° to 100 ml. Determine the *specific gravity* at 25°, Appendix 5.19. Find the percentage v/v of ethyl alcohol at 15.56° in the preparation by reference to the accompanying Table 1. If the specific gravity is found to be between two values, the percentage of alcohol should be obtained by interpolation.

NOTES – (1) If excessive frothing is encountered during distillation, render the solution strongly acid with phosphoric acid or treat with a small amount of liquid paraffin or a silicone oil.

(2) The distillate should be clear or not more than slightly cloudy. If it is turbid or contains oily drops, proceed by Method III. When steam-volatile acids are present, make the solution just alkaline with N sodium hydroxide using solid phenolphthalein as indicator before distillation.

Method II

This method and Method III are suitable for preparation that containing appreciable proportions of volatile materials other than alcohol and water.

Mix 25 ml of the sample, accurately measured at 25°, with about 100 ml of *water* in a separator. Saturate this mixture with *sodium chloride*, then add about 100 ml of *hexane*, and shake the mixture vigorously for two to three minutes. Allow the mixture to stand for about 15 to 20 minutes. Run the lower layer into the distillation flask. Wash the hexane in the separator by shaking vigorously with about 25 ml of *sodium chloride solution* to stand

and run the wash liquor into the first saline solution. Make the mixed solutions just alkaline with *N sodium hydroxide*, using solid *phenolphthalein* as indicator, add a little pumice powder and 100 ml of *water*, distil 90 ml and determine the percentage v/v of ethyl alcohol by **Method I**, commencing at the words "Adjust the temperature. . . .".

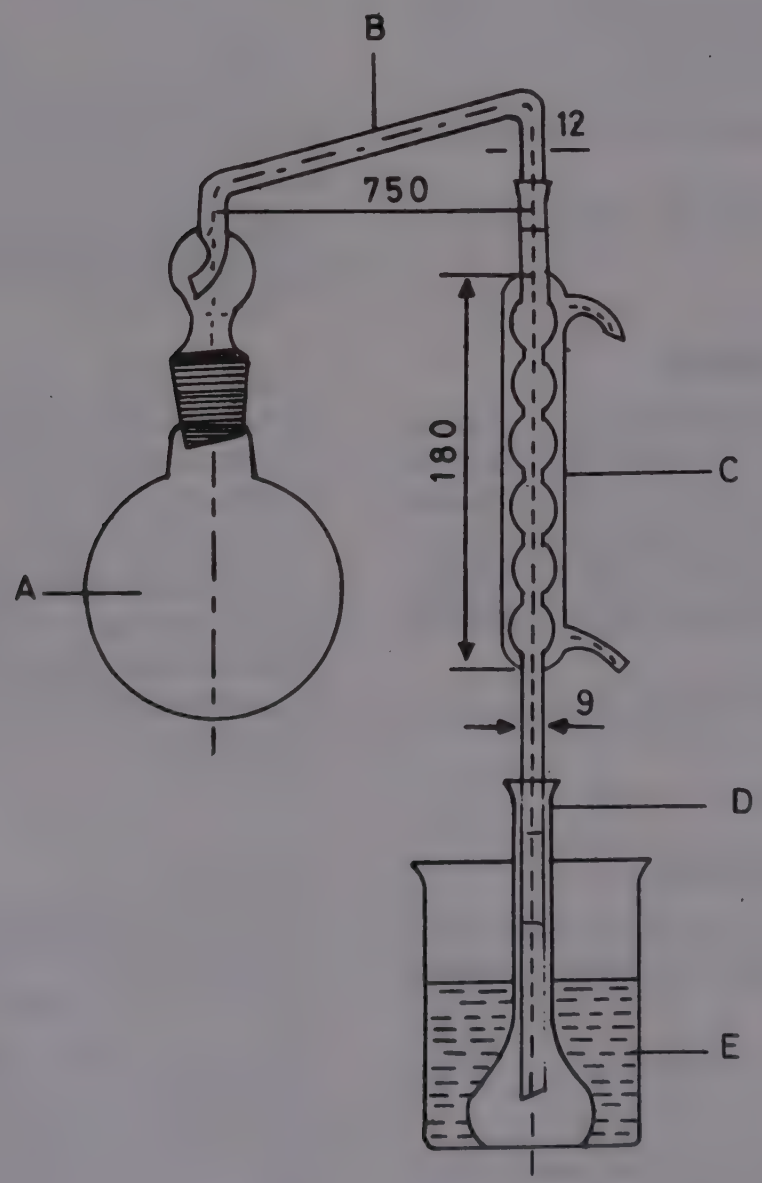
Method III

Transfer 25 ml of the sample, accurately measured, at 25° to the distillation flask. Dilute with 150 ml of *water* and add a little pumice powder. Attach the distillation head and condenser. Distil and collect about 100 ml. Transfer to a separator and determine the percentage v/v of ethyl alcohol by **Method II**, commencing at the words "Saturate this mixture.....".

The final distillate obtained by the above methods complies with the following tests:

(a) When adjusted to approximately 10 per cent alcoholic strength, either by dilution with *water* or by the addition of *alcohol (90 per cent)* 5 ml complies with the test for **Methyl Alcohol** described under Alcohol (absence of Industrial Methylated Spirit); the colour characteristic of the presence of formaldehyde, may be due to the presence in the original preparation of methyl compounds occurring naturally or produced during manufacture and in this case dose not necessarily denote the presence of Industiral Methylated Spirit. A determination of methyl alcohol will show whether any considerable amount of Industrial Methylated Spirit (which contains 5 per cent v/v of methyl alcohol) is present. Where Industrial Methylated Spirit has been employed in the formulation, test (a) will show the presence of methyl alcohol, as indicated by a violet-coloured solution of the same intensity as that produced when 0.5 ml of specially denatured spirit diluted with *water* to 5 ml is similarly treated.

(b) 1 ml mixed with 2 ml of *mercuric sulphate solution* and heated just to boiling-point gives no precipitate (absence of isopropyl alcohol).



(Dimensions in mm)

Fig. 1 DETERMINATION OF ALCOHOL (DISTILLATION METHOD)

TABLE 1

Specific Gravity at 25°	Ethyl Alcohol Content (per cent v/v of the Preparation at 15.56°	Specific Gravity at 25°	Ethyl Alcohol Content (per cent v/v of the Preparation at 15.56°
0.9695	96.88	0.9850	44.00
0.9700	95.20	0.9855	42.40
0.9705	93.52	0.9860	40.84
0.9710	91.84	0.9865	39.24
0.9715	90.12	0.9870	37.64
0.9720	88.44	0.9875	36.04
0.9725	86.72	0.9880	34.44
0.9730	85.00	0.9885	32.88
0.9735	83.28	0.9890	31.32
0.9740	81.52	0.9895	29.76
0.9745	79.80	0.9900	28.20
0.9750	78.00	0.9905	26.68
0.9755	76.24	0.9910	25.16
0.9760	74.48	0.9915	23.64
0.9765	72.72	0.9920	22.16
0.9770	70.96	0.9925	20.68
0.9775	69.20	0.9930	19.24
0.9780	67.48	0.9935	17.80
0.9785	65.76	0.9940	16.36
0.9790	64.00	0.9945	14.96
0.9795	62.32	0.9950	13.56
0.9800	60.60	0.9955	12.16
0.9805	58.88	0.9960	10.80
0.9810	57.20	0.9965	9.40
0.9815	55.52	0.9970	8.04
0.9820	53.84	0.9975	6.68
0.9825	52.16	0.9980	5.36
0.9830	50.52	0.9985	4.00
0.9835	48.88	0.9990	2.64
0.9840	47.24	0.9995	1.32
0.9845	45.64	1.0000	0.00

B. Gas-Liquid Chromatographic Methods

The following gas-liquid chromatographic methods are to be used where specified in the individual monograph.

Method I

Carry out the method for *gas-liquid chromatography*, Appendix 5.4.1, using the following solutions : (1) a solution containing 5.0 per cent v/v of *ethyl alcohol* and 5.0 per cent v/v of *propyl alcohol* (internal standard), (2) a volume of the preparation diluted with *water* to contain between 4.0 and 6.0 per cent v/v of ethyl alcohol, and (3) solution (2) containing 5.0 per cent v/v of the internal standard. The chromatographic procedure may be carried out using (a) a column 1.5 m long and 4 mm in internal diameter packed with porous polymer beads (100 to 120 mesh), maintained at 150° (either Porapak Q or Chromosorb 101 is suitable), (b) nitrogen as the carrier gas, and (c) a flame ionisation detector; maintain the injection port and the detector at 170°.

Method II

For preparations in which, in accordance with the authority given in the monographs, Industrial Methylated Spirit has been used, determine the content of ethyl alcohol and methyl alcohol, respectively as described in Method I, using for solution (2) a volume of the preparation diluted with *water* to contain between 4.0 and 6.0 per cent v/v of combined ethyl alcohol and methyl alcohol. In addition, prepare a graph from a solution (4) containing 0.25 per cent v/v of *methyl alcohol* and 5.0 per cent v/v of the internal standard, increasing the amplifier gain when recording the methyl alcohol peak in order to obtain a response comparable with that of the internal standard. When examining solution (3), similarly increase the amplifier gain when recording the peak due to the methyl alcohol. The total alcohol content is within the range specified in the monograph, and the ratio of the content of methyl alcohol to that of ethyl alcohol is commensurate with Industrial Methylated Spirit having been used.

5.3 DETERMINATION OF BOILING OR DISTILLING RANGE

The boiling or distilling range of a liquid is the temperature interval, corrected for a pressure of 760 torr within which the liquid, or a specified fraction of the liquid, distils under the conditions specified in the test. The lower limit of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser, and the upper limit is the temperature at which the last drop evaporates from the lowest point in the distillation flask without taking into account any liquid remaining on the sides of the flask; it may also be the temperature observed when the proportion specified in the individual monograph has been collected.

Apparatus

Use an apparatus consisting of the following :

1. Distilling flask : A round-bottom distilling flask of 200-ml capacity and having a total length of 17 to 19 cm and an inside neck diameter of 20 to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side-arm 10 to 12 cm long and 5 mm in internal diameter which is at an angle of 70° to 75° with the lower portion of the neck.

2. Condenser : A straight glass condenser 55 to 60 cm long with a water-jacket about 40 cm long or any other type of condenser having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or it may be connected to a bent adaptor that serves as a delivery tube.

3. Receiver : A 100-ml cylinder, graduated in 1-ml subdivisions.

4. Thermometer : An accurately standardised, partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°). When placed in position, the stem is located in the centre of the neck and the top of the bulb is just below the bottom of the outlet to the side-arm.

Method

If the liquid under examination distils below 80°, cool it to between 10° and 15° before measuring the sample for distillation.

Assemble the apparatus, and place in the flask 100 ml of the liquid under examination, taking care not to allow any of the liquid to enter the side-arm. Insert the thermometer and shield the entire heating and flask assembly from external air currents. Add a few pieces of porous material and heat rapidly to boiling using a Bunsen burner and an asbestos plate pierced by a hole 33 mm in diameter. Record the temperature at which the first drop of distillate falls into the cylinder, and adjust the rate of heating to obtain a regular distillation rate of 4 to 5 ml per minute. Record the temperature when the last drop of liquid evaporates from the bottom of the flask or when the specified percentage has distilled over. Correct the observed temperature readings for any variation in the barometric pressure from the normal (760 torr) using the following expression;

$$t_1 = t_2 + k\ (a - b)$$

where

t_1 = the corrected temperature

t_2 = the observed temperature

a = 760 (torr)

b = the Barometric pressure in torr at the time of determination

k = the correction factor indicated in the following table :

Distillation range	k
Less than 100°	0.040
100° to 140°	0.045
140° to 190°	0.050
190° to 240°	0.055
More than 240°	0.060

5.4 CHROMATOGRAPHY

5.4.1 Gas-liquid Chromatography (Gas Chromatography)

Gas chromatography is a technique for separation of mixtures into fractions by a process which depends on the redistribution of the components of the mixture between a stationary phase in the form of a liquid, solid or combination of both and a moving phase in the form of a gas.

Apparatus

A suitable gas chromatograph consisting of following components:

(a) A chromatographic column, usually of glass, stainless steel or copper, packed with a suitable finely divided inert solid support coated with a stationary phase. The column should be cured by being operated until stable at a temperature higher than that specified for use in the individual monograph.

(b) A sample injection port leading to the chromatographic column. The injection port must be heated to a temperature high enough to ensure rapid vaporisation of the substance being chromatographed but not to cause thermal degradation.

(c) A carrier gas flowing at a constant rate to the sample injection port and then on through the packed column to the detector.

(d) A suitable detector into which the components emerging individually from the end of the chromatographic column enter. The temperature of the detector must be controlled to prevent condensation.

(e) An automatic recording device to which the detector is coupled. A recording potentiometer is suitable. The resulting record is a signal time plot which is used to determine the identities and concentrations of the components. The length of the chromatographic column, the stationary phase, the solid support, the temperature, the carrier gas, and the detector suitable for the determination are stated in the individual monograph.

Samples for test – These are usually in the form of solutions called solutions (1), (2) and (3) in the individual monograph. The samples are usually injected by a syringe through a rubber septum in the injection port and directly into the column packing so that its vapour mixes instantaneously with the flowing carrier gas and is swept into the column.

Method

Using solution (1), determine experimentally suitable sensitivity setting of the instrument and the volumes of solutions to be injected to produce an adequate response. Prepare a chromatogram by injecting the selected volume of solution (1) through the sample injection port on the chromatographic column, maintained at a suitable temperature, and eluting with the carrier gas. Repeat the determination twice more. In the same manner prepare graphs using the same volumes of solutions (2) and (3). Measure the peak areas or alternatively, where the symmetry factor lies between 0.95 and 1.05, the peak heights produced by the substance or substances under test and the internal standard. If an impurity peak has the same retention time as the internal standard, allowance is made in assessing the responses of these constituents for the contribution of each. From the values obtained calculate the proportion of the substance or substances being examined.

The symmetry factor of a peak is calculated from the expression $Y_x/2A$, where Y_x is the width of the peak at one-twentieth of the peak height; A is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Unless otherwise stated in the monograph, the resolution factor (R) between measured peaks on the chromatogram must be at least 1.0 and is defined by the expression:

$$R = 2(V_{Rb} - V_{Ra})/(Y_a + Y_b),$$

where

V_{Ra} and V_{Rb} = the distances along the baseline between the point of injection and perpendiculars dropped from the maxima of two adjacent peaks.

Y_a and Y_b = the respective baseline peak widths.

The values of Y_x, Y_a, Y_b, V_{Ra} and V_{Rb} must be expressed in the same unit of measurement.

5.4.2 Paper Chromatography

Paper chromatography is a technique in which separation of the components of a mixture is achieved through the action of a single liquid phase in a process similar to adsorption chromatography in columns and in which a

sheet of paper of suitable texture and thickness acts as the adsorbent. Since the natural water content of the paper, or selective inhibition of a hydrophilic component of the liquid phase by the paper may be regarded as a stationary phase, a partitioning of the solute between two phases may contribute to the separation of components.

Alternatively, a two-phase system may be adopted in which the paper is impregnated with one of the phases, which then remains stationary and the chromatogram is developed by slow movement of the other, mobile, phase over the sheet. Development may be ascending in which case the mobile phase is carried up the paper by capillary forces, or descending in which case the mobile phase is also assisted by gravitational force.

Apparatus

(a) A vapour-tight tank of glass, porcelain or stainless steel provided with inlets for addition of solvent or for releasing internal pressure and so designed that the progress of the chromatographic run can be observed without opening the tank.

(b) A rack of corrosion-resistant material about 5 cm shorter than the inside height of the tank, to serve as a support for the solvent trough and for antisiphoning rods which in turn, hold the chromatographic sheets.

(c) Glass troughs, longer than the width of the chromatograph sheets and holding a volume of solvent greater than that needed for one chromatographic run.

(d) Antisiphoning rods of heavy glass to be supported by the rack, and running outside of, parallel to, and slightly above the edge of the glass trough.

(e) Chromatographic sheets of special filter paper not less than 25 mm wide and not wider than the length of the troughs, cut into strips to a length approximately equal to the height of the tank; the paper is cut so that the mobile phase runs in the direction of the grain of the paper. A fine pencil line is drawn horizontally across the filter paper at a distance from one end such that, when the sheet is suspended from the anti-siphoning rods with the upper end of the paper resting in the trough and the lower portion hanging free into the chamber, the line is located a few cm below the rods. Care should be taken to avoid contaminating the filter paper by excessive handling or by contact with dusty surfaces.

Descending Paper Chromatography

Method : The substance being examined is dissolved in a suitable solvent. The volumes of the resulting solution are applied by means of a micropipette in 6 to 10 mm spots along the pencil line not less than 3 cm apart. If the total volume to be applied would produce spots of a diameter greater than 6 to 10 mm, it is applied in separate portions to the same spot, each portion being allowed to dry before the next is added.

Use sufficient of the saturating solvent prescribed in the monograph, to form a 25 mm layer in the bottom of the tank. Close the tank, allow to stand for twenty-four hours, at room temperature.

Suspend the spotted chromatographic sheet in the tank by the use of the antisiphoning rod, which holds the upper end of the sheet in the solvent trough. It is important to ensure that the portion of the sheet hanging below the rods is freely suspended in the tank without touching the rack or the tank walls or the fluid in the tank.

Close the tank and allow the paper to stand in it for one and a half hours. Introduce through the inlet into the solvent trough a sufficient quantity of the prescribed mobile phase, close the tank and allow development to proceed for the distance or the time prescribed in the monograph, protecting the paper from bright light during the elution process. Remove the paper from the tank and allow it to dry in air at the temperature specified in the monograph. Visualise the spots as described in the individual monograph. The paper section(s) (e-under Apparatus) predetermined to contain the separated components may be cut out and eluted by an appropriate solvent, and the solutions may be made up to a known volume and quantitatively analysed by appropriate chemical of instrumental methods.

Ascending Paper Chromatography

Method : The test materials are applied to the chromatographic sheets as directed under **Descending Paper Chromatography**, and above the level to which the paper is dipped into the developing solvent. The top of the tank contains a device from which the paper is suspended, and which is capable of being lowered without opening the tank. In the bottom of the tank is a trough to contain the mobile phase, into which the paper may be lowered.

Use sufficient of the prescribed mobile phase to form a 25 mm layer in the trough. If a two-phase system is used, both phases are added. If prescribed pour the saturating solvent between the trough and the walls of the tank. Close the tank and allow to stand for twenty-four hours, at room temperature. Insert the prepared paper into the tank, close the lid and allow to stand for one and a half hours. Lower the paper into the mobile phase and allow development to proceed for the distance or the time prescribed in the monograph, protecting the paper from bright light during the elution process. Remove the paper from the tank and allow to dry in air at the temperature specified in the monograph. Qualitative and quantitative analyses of the spots may be conducted as described under **Descending Paper Chromatography.**

5.4.3 Thin-layer Chromatography

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile

phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvents.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus

(a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions. The plates are prepared as described below; alternatively, commercially prepared chromatoplates may be used.

(b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

(c) The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 μm to 40μm in diameter, suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (hydrated calcium sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.

(d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

(e) A storage rack to support the plates during drying and transportation.

(f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber if fitted with a plate-support rack that supports the plates, back to back, with the lid of the chamber in place.

(g) Graduated micro-pipettes capable of delivering quantities of 10 μl and less.

(h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

(i) An *ultra-violet lamp*, suitable for observation at short (254 nm) and long (366 nm) ultra-violet wavelengths.

Preparation of plates : Unless otherwise specified in the monograph, the plates are prepared in the following manner: Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using a spreading device designed for the purpose, spread a uniform layer of the suspension 0.25 to 0.30 mm thick on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100° to 105° for at least one hour (except in the case of plates prepared with cellulose when ten minutes, heating is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within three days of preparation. At the time of use, re-dry the plates, if necessary, as prescribed in the monograph.

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for one hour at room temperature.

Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (20 mm × about 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the chromatoplate in the tank, ensuring that it is as nearly vertical as possible and that the spots are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

5.5. DETERMINATION OF CONGEALING RANGE OR TEMPERATURE

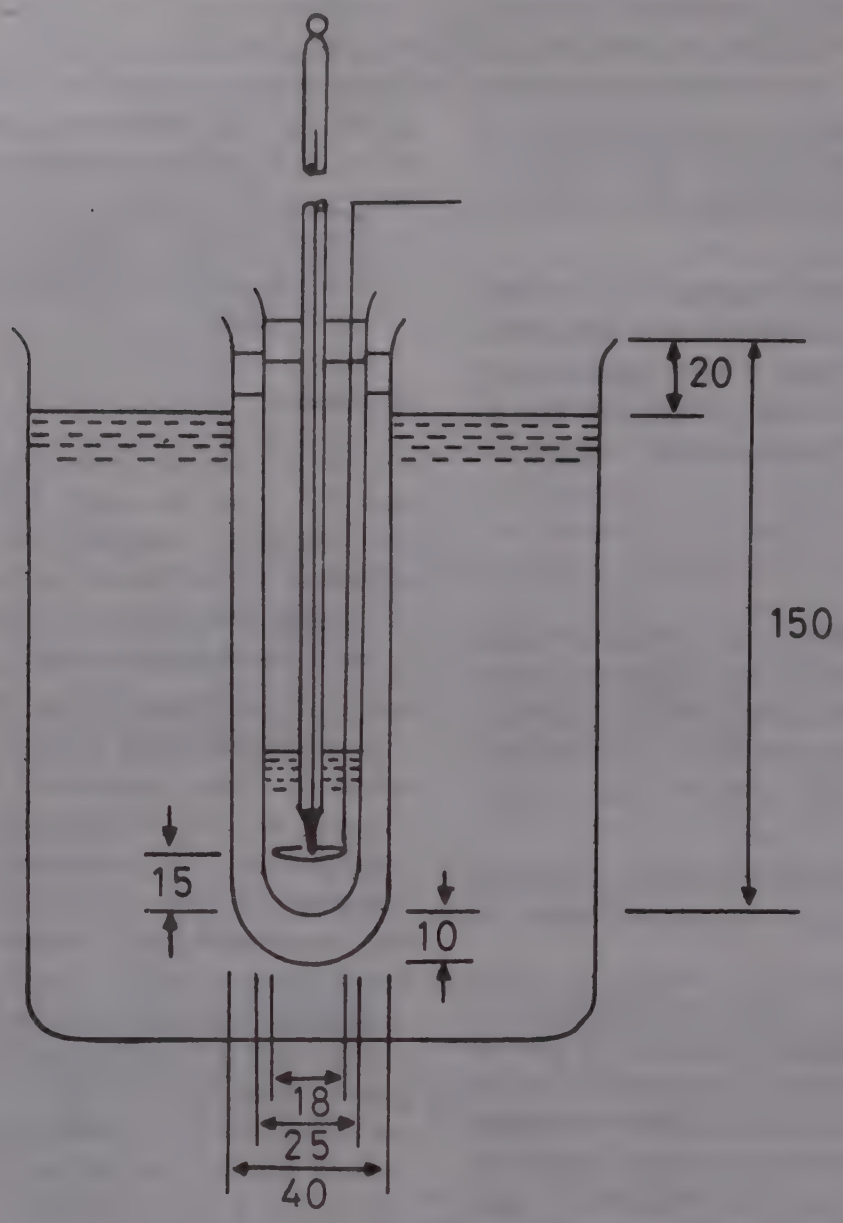
The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and a small but increasing proportion of the solid phase. It is distinct from the freezing point, which is the temperature at which the liquid and solid phases of a substance are in equilibrium.

The temperature at which a substance solidifies upon cooling is a useful index of its purity if heat is liberated when solidification takes place.

The following method is applicable to substances that melt between −20° and 150°

Apparatus

A test-tube about 25 mm in diameter and 150 mm long



(Dimensions in mm)

Fig. 2

placed inside a test-tube about 40 mm in diameter and 160 mm long; the inner tube is closed by a stopper that carries a stirrer and a thermometer (about 175 mm long and with 0.2° graduations) fixed, so that the bulb is about 15 mm above the bottom of the tube. The stirrer is made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1-litre beaker containing a suitable cooling liquid to within 20 mm of the top. A thermometer is supported in the cooling bath (*see* Fig. 2).

Method

Melt the substance, if a solid, at a temperature not more than 20° above its expected congealing point, and pour it into the inner test-tube to a height of 50 to 57 mm. Assemble the apparatus with the bulb of the thermometer immersed half-way between the top and bottom of the sample in the test-tube. Fill the bath to almost 20 mm from the tube with a suitable fluid at a temperature 4° to 5° below the expected congealing point. If the substance is a liquid at room temperature, carry out the determination using a bath temperature about 15° below the expected congealing point. When the sample has cooled to about 5° above its expected congealing point stir it continuously by moving the loop up and down between the top and bottom of the sample, at a regular rate of 20 complete cycles per minute. Record the reading of the thermometer every 30 seconds and continue stirring only so long as the temperature is falling. Stop the stirring when the temperature is constant or starts to rise slightly. Continue recording the temperature for at least three minutes after the temperature again begins to fall after remaining constant.

The congealing point will be the average of not less than four consecutive readings that lie within a range of 0.2°.

5.6 DISINTEGRATION TEST

5.6.1 Disintegration Test for Tablets

This test is provided to determine whether uncoated and coated tablets disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions. The test is not applicable to sustained release tablets.

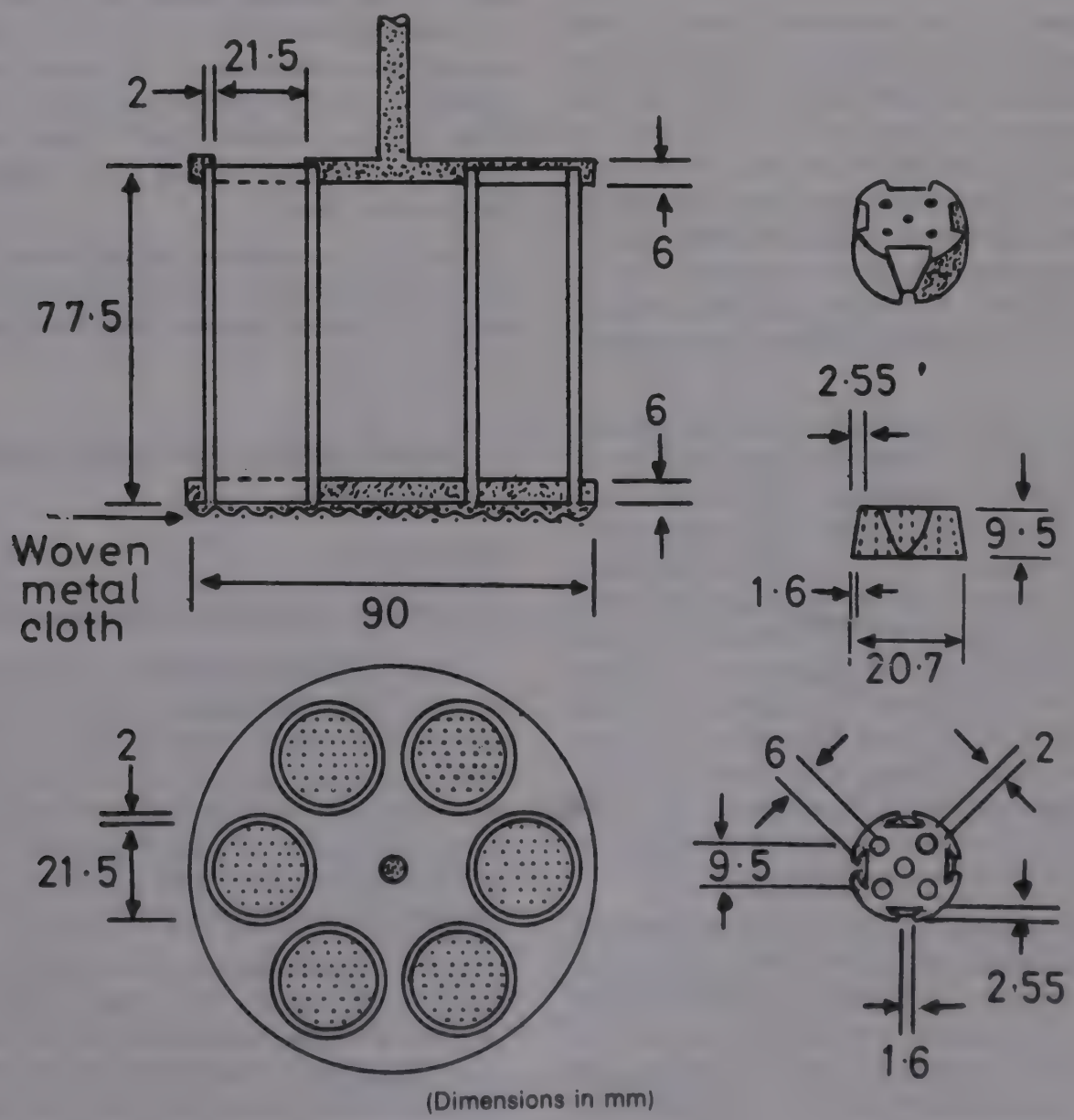
For the purposes of this test, disintegration does not imply complete solution of the tablet or even its active constituent. Disintegration is defined as that state in which any residue of the tablet, except fragments of insoluble coating remaining on the screen of the test apparatus consists of a soft mass having no palpably firm, unmoistened core.

Apparatus

(a) A rigid basket-rack assembly supporting six cylindrical glass tubes 77.5 mm long, 21.5 mm in internal diameter, and with a wall thickness of about 2 mm.

(b) The tubes are held vertically by two superimposed transparent plastic plates 90 mm in diameter and 6 mm thick, perforated by six holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the under side of the lower plate is a piece of woven gauze made from stainless steel wire 635 μm in diameter and having nominal mesh apertures of 2.00 mm. The upper plate is covered with a stainless steel disc perforated by six holes each about 22 mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper open ends of the glass tube.

(c) The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 28 and 32 cycles per minute, through a distance of 50 to 60 mm (*see* Fig. 3).



(Dimensions in mm)

Fig. 3

The design of the basket-rack assembly may be somewhat different provided specifications for the glass tubes and the screen mesh size are unchanged.

(d) a cylindrical disc for each tube, each 20.7 mm in diameter and 9.5 mm thick, made of transparent plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2 mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6 mm from the centre of the disc. Four equally-spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5 mm wide and 2.55 mm deep and at the lower surface 1.5 mm square.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferably a 1000-ml beaker. The volume of liquid is such that the wire mesh at its highest point is at least 25 mm below the surface of the liquid, and at its lowest point is at least 25 mm above the bottom of the beaker.

(f) A thermostatic arrangement for heating the liquid and maintaining the temperature between 35° and 39°.

Methods

(1) Uncoated tablets : Unless otherwise stated in the individual monograph place one tablet in each of the six tubes of the basket, add a disc to each tube, and operate the apparatus, using *water* maintained at 37° ± 2° as the immersion liquid. At the end of fifteen minutes or the times specified in the individual monograph, lift the basket from the liquid and observe the tablets. The tablets pass the test if all six have disintegrated. If one or two tablets fail to disintegrate, repeat the test on twelve additional tablets; not less than sixteen of the total of eighteen tablets tested disintegrate.

(2) Coated tablets : Place one tablet in each of the six tubes of the basket, and if the tablet has a soluble external coating, immerse the basket in *water* at room temperature for five minutes. Then add a disc to each tube, suspend the assembly in *water* maintained at 37° ± 2° and operate the apparatus for sixty minutes, unless otherwise indicated in the individual monograph. The tablets pass the test if all six have disintegrated. If any of the tablets have not disintegrated, repeat the test on a further six tablets, replacing *water* with *0.1N hydrochloric acid*. The tablets then pass the test if all six tablets have disintegrated in the acid medium. If one or two tablets still fail to disintegrate, repeat the test on twelve additional tablets; not less than sixteen of the total of eighteen tablets tested disintegrate.

(3) Enteric-coated tablets : Place one tablet in each of the six tubes of the basket, and if the tablet has a soluble external coating, immerse the basket in *water* at room temperature for five minutes. Operate the apparatus, without adding the discs, using *0.1N hydrochloric acid* as the immersion liquid maintained at 37° ± 2°. At the end of two hours, remove the assembly from the liquid. No tablet shows signs of either disintegration, apart from fragments of coating, or cracks that would allow the escape of the contents. Then add a disc to each tube and operate the apparatus using *mixed phosphate buffer pH 6.8* as the immersion liquid maintained at 37° ± 2°. At the end of sixty minutes remove the assembly from the liquid and observe the tablets. The tablets pass the test if all six have disintegrated. If one or two tablets fail to disintegrate, repeat the test on twelve additional tablets; not less than sixteen of the total of eighteen tablets tested disintegrate.

5.6.2 Disintegration Test for Capsules

Apparatus

Use the apparatus described under *disintegration test for tablets*, Appendix 5.6.1.

Method

Place one capsule in each of six tubes of the basket and suspend the assembly in *water* maintained at 37° ± 2°. Omit the discs for hard capsules except that if the hard capsules float on top of the water the discs may be added. When testing soft capsules discs may be used. Operate the apparatus for thirty minutes in the case of hard capsules and for sixty minutes in the case of soft capsules. Remove the assembly from the liquid. The capsules pass the test if no residue remains on the screen of the apparatus or, if a residue remains, it consists of fragments of shell or is a soft mass with no palpable core. If discs have been used, any residue remaining on their lower surface consists only of fragments of shell.

If one or two capsules fail to disintegrate, repeat the test on twelve additional capsules; not less than sixteen of the total of eighteen capsules tested disintegrate.

5.7 DISSOLUTION TEST FOR TABLETS AND CAPSULES

Apparatus

An assembly consisting of the following:

(a) A cylindrical covered, 1000-ml vessel made of glass or other inert, transparent material (which does not react or interfere with or absorb the substance being tested), with an internal diameter of 100 mm and a flanged upper rim. The vessel may be flat-bottomed or have a spherical bottom. It is 60 to 175 mm high and has a nominal capacity of 1000 ml. The vessel is fitted with a lid which has a number of openings, one of which is central.

(b) A variable-speed motor which causes a basket to rotate in the vessel. The speed of the motor should be capable of being varied between 25 and 150 revolutions per minute and maintained within 5 per cent of the required speed. The motor drives the basket by means of a shaft, approximately 6 mm to 10.5 mm in diameter and about 300 mm long. It is so positioned that its axis is not more than 2 mm at any point from the vertical axis of the

vessel and enables the basket to revolve smoothly and without perceptible wobble. The shaft passes through the central opening in the lid of the vessel and carries at its lower and a stainless steel disc of 25 mm diameter in which there is a 2 mm vent.

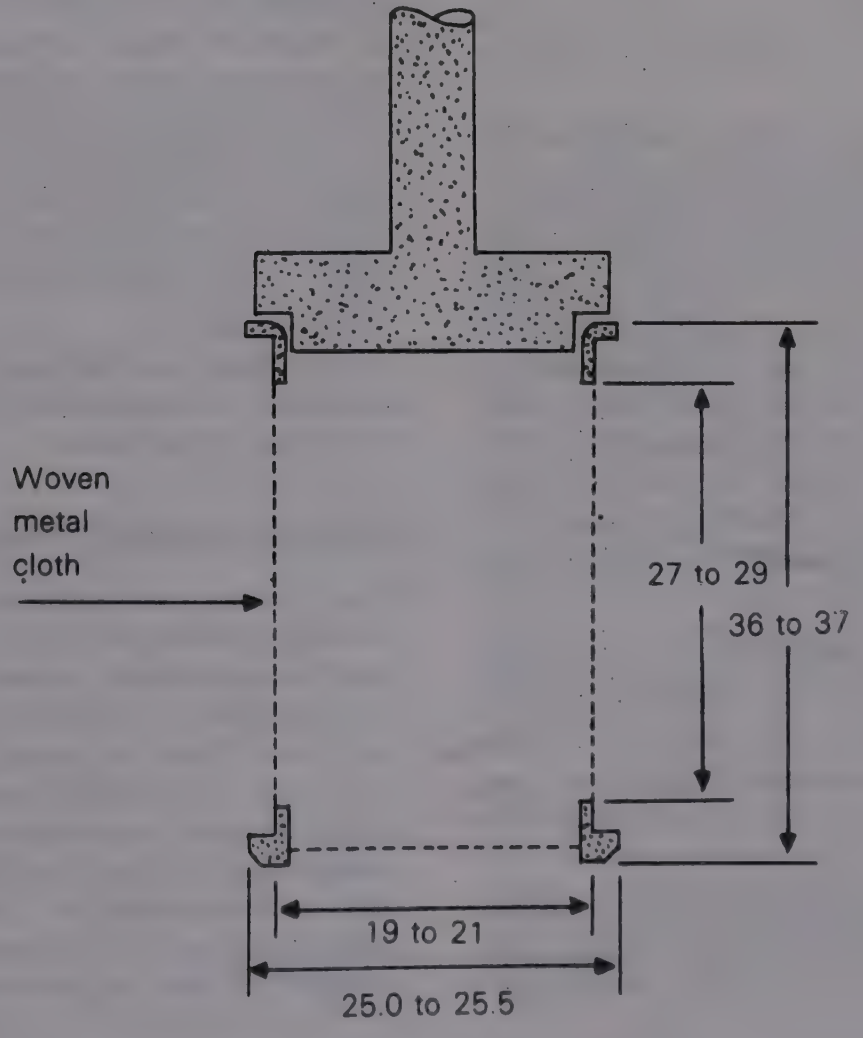
(c) A cylindrical stainless steel basket assembly made of woven wire cloth with a nominal aperture size of 425 µm, joined down one side with a narrow welded seem and with a flanged rim at each end (*see* Fig. 4). For use with dilute acid media the basket may be coated with not more than a 2.5 µm thickness of gold. The top of the basket is attached to the disc on the driving shaft by three clips or by other suitable means.

(d) The vessel is equipped with a suitable device for the withdrawal of samples of the dissolution medium from a point approximetely half-way between the basket wall and the wall of the vessel and half-way between the surface of the dissolution medium and the bottom of the vessel. The method of sampling should not interfere excessively with the normal pattern of flow of the dissolution medium in the apparatus.

(e) The vessel should be securely clamped in a water-bath in such a way that the displacement vibration from other equipment, including the water-circulating device, is minimised. The water-bath is set to maintain the temperature of the dissolution medium in the vessel at 37° ± 0.5°. The bath liquid must be kept in constant, smooth motion during the test.

Method

Unless otherwise stated in the monograph, introduce into the vessel 1000 ml of *water*, free from dissolved air and previously warmed to 36.5° to 37.5°. Place the specified number of tablets or capsules in the dry basket, assemble the apparatus, adjusting the distance between the bottom of the basket and the bottom interior surface of the vessel to between 23 mm and 27 mm. Start the motor and adjust the rotational speed to 100 rpm or such other speed as may be indicated in the monograph. Withdraw the stated volume of the solution from the vessel at forty-five minutes or at the time or times specified; filter immediately through an inert medium with a nominal pore size of



Details of the Basket Assembly

(Dimensions in mm)

Fig 4 DISSOLUTION TEST FOR TABLETS AND CAPSULES

1 μm or less (such as Millipore TF). Determine the amount of active ingredient present by the method given in the monograph. Repeat the complete operation four times.

Interpretation – The requirements of the test are met if, for each of the five tablets or capsules tested (in those cases where one tablet or capsule is directed to be placed in the basket for each test) the amount of active ingredient in solution is not less than 70 per cent of the stated amount. Where two or more tablets or capsules are directed to be placed together in the basket for each test in the five replicate tests, the amount of active ingredient in solution per tablet or capsule in each test is not less than 70 per cent of the stated amount, unless otherwise specified in the monograph. No re-testing is permitted.

5.8 LOSS ON DRYING

The following procedure determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

Loss on drying is the loss in weight in per cent w/w determined by means of the procedure given below. Unless otherwise directed in the monograph, carry out the test on 1.0 g of the substance, previously mixed well. If the sample is in the form of large crystals, reduce the size by quickly crushing to a powder.

Method

Weigh a glass-stoppered, shallow weighing bottle that has been dried for 30 minutes under the same conditions to be employed in the determination. Put the sample in the bottle, cover it and accurately weigh the bottle and the contents. Distribute the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm. Place the loaded bottle in the drying chamber (oven or desiccator), remove the stopper and leave it also in the chamber. Dry the sample for the time specified in the monograph or to constant weight,* at the prescribed temperature under one of the following conditions (Shown in brackets are the words used in the individual monograph for the drying conditions).

(a) In a desiccator over phosphorous pentoxide at atmospheric pressure and at room temperature ("desiccator").

(b) Over phosphorous pentoxide, in vacuum at a pressure not exceeding 20 Torr at room temperature ("in vacuo").

(c) Over phosphorous pentoxide, in vacuum at a pressure not exceeding 20 Torr at a higher temperature ("in vacuo with indication of temperature and time").

(d) In an oven at the temperature indicated in the monograph.

After drying is completed, open the drying chamber, close the bottle promptly and allow it to come to room temperature (where applicable) in a desiccator before weighing.

NOTE – Where drying in a desiccator is specified, care must be taken to keep the desiccant fully effective by frequent replacement.

5.9 ELECTROPHORESIS

Electrophoresis is a physical method of analysis involving the separation of compounds that are capable of acquiring an electrical charge in a conducting electrolyte. In this medium the charged particles which are dissolved or dispressed more or less rapidly under the influence of an electric field.

The electrophoretic mobility is the rate of movement of the substance in metres per second of the charged particles under the action of an electric field of 1 volt per metre. For practical reasons it is expressed in square centimetres per volt second ($cm^2.V^{-1}s^{-1}$).

The mobility can be defined only for a given electrolyte under precisely determined operational conditions. It is influenced by many factors such as the characteristics of the substance, its nature, size, form, electrical charge and friction coefficient. It also depends on the composition of the conducting liquid, its nature, concentration, pH, the presence of additional solvents and viscosity. The direction of movement depends on the sign of the electrical charge of the particle as it moves towards the electrode of opposite sign.

Depending on the method used, the electrophoretic mobility is either measured directly or compared with that of a reference substance.

Moving Boundary (Free-flow) Electrophoresis

This method, used exclusively for the determination of mobility, has the advantage of the experimental characteristics being directly measurable and reproducible. It is chiefly employed for substances of high molecular weight with poor diffusion properties. The boundaries are initially located by a physical process such as refractometry or conductimetry. After applying a given electric field for an accurately measured time, the new boundaries and their respective positions are observed. The operating conditions must permit the determination of as many boundaries as there are components.

Zone Electrophoresis

In this method a supporting medium is used and sample sizes are very small. The nature of the supporting medium such as paper, agar gel, cellulose acetate, starch-gel, poly-

* The term 'constant weight' means that two consecutive weighings do not differ by more than 0.5 mg; the second weighing being made after an additional one hour of drying under the specific conditions.

methacrylamide, mixed gel, introduces additional factors influencing the mobility. The rate of migration depends on four main factors namely, the mobility of the particles, the electro-endosmotic current (in the case of carriers with polar properties), the evaporation flow (caused by heat generated through the Joule effect) and on the gradient of the electric field. Hence it is necessary to operate under clearly defined experimental conditions and to use, to the extent possible, reference substances.

In practice, the mobility of the electrophoretic zones and their signs are ignored; the zones are located by experience or by comparison with those given by a reference substance treated in the same way. Colourless substances may be located and determined by treating the electrophorogram with a reagent that will convert them to coloured or fluorescent derivatives. For quantitative estimations, the zones may be carefully separated, the substance eluted with a suitable solvent and then determined by a sufficiently sensitive method, such as a spectrophotometric measurement, either directly or after a chemical reaction. Alternatively, after conversion to a coloured derivative, the zone colour intensity can be measured with a scanning densitometer.

An apparatus for electrophoresis on a supporting medium consists of:

(1) A source of direct current, preferably of stabilised voltage;

(2) An electrophoresis chamber, generally rectangular and made of glass or rigid plastic material with an air-tight lid which maintains a moisture saturated atmosphere during operation and reduces evaporation of the solvent. A safety device may be used to cut off the power when the lid is removed. The chamber should have two compartments, the anodic and the cathodic, containing the electrolyte solution. In each compartment is immersed an electrode, for example of platinum or graphite which are connected through external insulated cables to an electrical power source having an output of not less than 450 V DC at 150 mA. The power source should be provided with a means of indicating and controlling the voltage and of indicating the current consumption. If the electrical power measured across the strip exceeds 10W, it is advisable to cool the support. The level of the liquid in the two compartments is kept equal to prevent siphoning.

(3) A support-carrying device when paper or cellulose acetate is used, the carrier strips impregnated with the conducting solution are stretched by a suitable arrangement and the ends immersed into the electrode compartments. In gel electrophoresis, the device consists of a glass plate over the whole surface of which is deposited a firmly adhering layer of gel of uniform thickness and at each end of which are attached the electrical connections. In using these devices precautions must be taken to avoid condensation of moisture or drying of the solid layer.

(4) A measuring device or means of location and measurement of spots (zones).

Method

Introduce the electrolyte solution into the electrode compartments and place the support suitably impregnated with electrolyte in the chamber under the conditions prescribed for the apparatus. Locate the starting line and apply the sample. Apply the electric current for the prescribed time. Switch off the current, remove the support from the chamber, dry and measure the spots by a suitable method.

5.10 DETERMINATION OF pH VALUES

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In the pharmacopoeia, standards and limits on pH have been provided for those pharmacopoeial substances in which pH as a measure of the hydrogen-ion activity is important from the standpoint of stability or physiological suitability.

The measurement of pH is generally done with a suitable potentiometric meter known as the pH meter fitted with two electrodes, one constructed of glass and sensitive to hydrogen-ion activity and the other a calomel reference electrode. The determination is carried out at a temperature of 25° ± 2°, unless otherwise specified in the individual monograph.

Apparatus

The pH value of a solution is determined potentiometrically by means of a glass electrode, a reference electrode and a pH meter either of the potentiometric or of the deflection type.

Operate the pH meter and electrode system according to the manufacturer's instructions. Calibrate the apparatus using *buffer solution D* as the primary standard, adjusting the meter to read the appropriate pH value given in the Table 1, corresponding to the temperature of the solution. Where provision is made for setting the scale, use a second reference buffer solution, either *buffer solution A*, *buffer solution E* or *buffer solution G*. In this case a check is carried out with a third reference buffer solution of intermediate pH, when the reading of the intermediate solution must not differ by more than 0.05 pH unit from the corresponding value indicated in the Table. Where there is no provision for setting the scale with a second reference buffer solution, checks should be made with two reference buffer solutions, the readings for which must not differ by more than 0.05 pH unit from the value corresponding to each solution.

Reference Buffer Solutions

The following reference buffer solutions must be prepared

TABLE 1 – pH of Reference Buffer Solutions at Various Temperatures

Temperature	Buffer Solutions							
t^0	A	B	C	D	E	F	G	H
15	1.67	–	3.80	4.00	6.90	7.45	9.28	10.12
20	1.68	–	3.79	4.00	6.88	7.43	9.22	10.06
25	1.68	3.56	3.78	4.01	6.86	7.41	9.18	10.01
30	1.68	3.55	3.77	4.01	6.85	7.40	9.14	9.97
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93
$\Delta pH/\Delta t$	+0.001	−0.001	−0.002	+0.001	−0.003	−0.003	−0.008	−0.009

using *carbon dioxide-free water*; phthalate and phosphate salts should be dried at 110° for two hours before use. Buffer-solutions should be stored in bottles made of alkali-free glass, and must not be used later than three months after preparation.

1. Buffer solution A : Dissolve 12.71 g of *potassium tetraoxalate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

2. Buffer solution B : A freshly prepared saturated solution, at 25°, of *potassium hydrogen tartrate*.

3. Buffer solution C : Dissolve 11.51 g of *potassium dihydrogen citrate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

NOTE – This solution must be freshly prepared.

4. Buffer solution D : Dissolve 10.21 g of *potassium hydrogen phthalate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

5. Buffer solution E : Dissolve 3.40 g of *potassium dihydrogen phosphate* and 3.55 g of *anhydrous disodium hydrogen phosphate*, both previously dried at 110° to 130° for two hours, in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

6. Buffer solution F : Dissolve 1.184 g of *potassium dihydrogen phosphate* and 4.303 g of *anhydrous disodium hydrogen phosphate*, both previously dried at 110° to 130° for two hours, in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

7. Buffer solution G : Dissolve 3.814 g of *borax* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

NOTE – This solution should be stored protected from carbon dioxide.

8. Buffer solution H : Dissolve 7.155 g of *sodium carbonate* and 2.10 g of *sodium bicarbonate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

Method

Immerse the electrodes in the solution to be examined and measure the pH at the same temperature as for the standard solutions. At the end of a set of measurements, take a reading of the solution used to standardise the meter and electrodes. If the difference between this reading and the original value is greater than 0.05, the set of measurements must be repeated.

When measuring pH values above 10.0 ensure that the glass electrode is suitable for use under alkaline conditions and apply any correction that is necessary.

All solutions of substances being examined must be prepared using *carbon dioxide-free water*.

5.11 DETERMINATION OF MELTING RANGE OR TEMPERATURE

In this Pharmacopoeia, melting range or temperature of a substance is defined as those points of temperature within which, or the point at which, the substance begins to coalesce and is completely melted except as defined otherwise for certain substance. The following procedures are suitable for the various substances described in

	Melting range
Vanillin	81 °– 83 °
Acetanilide	114 °– 116 °
Phenacetin	134 °– 136 °
Sulphanilamide	164.5 °– 166.5 °
Sulphapyridine	191 °– 193 °
Caffeine (dried at 100°)	234 °– 237 °

the Pharmacopoeia. Any other apparatus or method capable of the same accuracy may also be used. The accuracy should be checked frequently by the use of one of the following reference substances, that melts nearest to the melting range of the substance to be tested:

Unless otherwise specified in the individual monograph, Method I should be used.

Method I

Apparatus : (a) A glass heating vessel of suitable construction and capacity containing one of the following or any other suitable bath liquid, to a height of not less than 14 cm.

(i) Water for temperatures upto 60°

(ii) Glycerin for temperatures upto 150°.

(iii) Liquid paraffin for sufficiently high boiling range for temperatures upto 250°.

(iv) Sesame oil or a suitable grade of liquid silicone for temperatures upto 300°.

(b) A suitable stirring device, capable of rapidly mixing the liquids.

(c) An accurately standardised thermometer suitable for the substance under examination (see Appendix 1.3). The thermometer must be positioned in the bath liquid to its specified immersion depth and yet leave the bulb at about 2 cm above the bottom of the bath.

(d) Thin-walled capillary glass tubes of hard glass, about 12 cm long, with a well thickness of 0.2 to 0.3 mm and an internal diameter of 0.8 to 1.1 mm. The tubes should preferably be kept sealed at both ends and cut as required.

(e) Source of heat (open flame or electric heater).

Procedure : Reduce the substance to a very fine powder and, unless otherwise directed, dry it at a temperature considerably below its melting temperature or under reduced pressure over a suitable desiccant for not less than 16 hours. Introduce into a capillary glass tube, one end of which is sealed, a sufficient quantity of the dry powder to form a compact column about 3 mm high.

Heat the bath until the temperature is about 10° below the expected melting point. Remove the thermometer and quickly attach the capillary tube to the thermometer by wetting both with a drop of the liquid of the bath or otherwise and adjust its height so that the closed end of the capillary is near the middle of the thermometer bulb. Replace the thermometer and continue the heating, with constant stirring, sufficiently to cause the temperature to rise at a rate of about 3° per minute. When the temperature is about 3° below the lower limit of the expected melting range, reduce the heating so that the temperature rises at a rate of about 1° to 2° per minute. Continue the heating and note the temperature at which the column of the sample collapses definitely against the side of the tube at any point, when melting may be considered to have begun and note also the temperature at which the sample becomes liquid throughout as seen by the formation of a definite meniscus. The two temperatures fall within the limits of the melting range.

Method II

Apparatus : Use the apparatus described under Method I except that the glass capillary tube is open at both ends and has an internal diameter of 1.1 to 1.3 mm, an external diameter of 1.4 to 1.7 mm and a length of 50 to 60 mm.

Procedure : Rapidly melt the material to be tested, at a temperature not more than 10° above the point of complete fusion. Draw it into a capillary tube to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 hours, or in contact with ice for at least 2 hours. Attach the tube to the thermometer and adjust it so that the column of substance is in level with the thermometer bulb; suspend the thermometer in the heating vessel containing water at 15° so that the lower end of the column of the substance is 30 mm below the surface of the water and heat the water with constant stirring so that the temperature rises at the rate of 1° per minute. The temperature at which the partly melted substance is observed to rise in the capillary tube is the melting temperature.

Method III

Apparatus : (a) A glass boiling-tube, overall length, 110 mm, internal diameter, 25 mm.

(b) A cork about 25 mm long to fit into the boiling-tube, bored with a central hole to fit the standard thermometer and with a groove cut in the side.

(c) A glass beaker, of such a size that when the apparatus is assembled the boiling-tube can be immersed vertically to two-thirds of its length in the water in the beaker with its lower end about 2.5 cm above the bottom of the beaker.

(d) A stirrer or any other device which will ensure uniformity of the temperature throughout the water in the beaker.

(e) An accurately standardised thermometer suitable for the substance under examination (see Appendix 1.3).

(f) Suitable means of heating the water in the beaker.

Procedure : Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of about 90° Cool and allow the temperature of the molten substance to drop to a temperature of 8° to 10° above the expected melting point. Chill the bulb of the thermometer to 5°, wipe it dry and while it is still cold dip it in the molten substance so that the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for five minutes into a water-bath at a temperature not higher than 15°.

Fit the thermometer through the bored cork into the boiling-tube so that the lower part is 15 mm above the bottom of the tube. Suspend the tube in the beaker filled with water adjusted to about 15°, and raise the temperature of the bath at the rate of 2° per minute to 30°, then adjust the rate to 1° per minute and note the temperatures at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the substance. If the three readings differ by less than 1°, take the average of the three as the melting point. If they differ by more than 1°, make two additional determinations and take the average of the five readings.

5.12 OPTICAL ROTATION AND SPECIFIC OPTICAL ROTATION

Optical rotation 'α' is the property shown by certain substances of rotating the plane of polarisation of polarised light. Such substances are said to be optically active in the sense that they cause incident polarised light to emerge in a plane forming a measurable angle with the plane of the incident light. Where this effect is large enough for measurement, it may serve as the basis for identifying or assaying a substance.

The *optical rotation* of a substance is the angle through which the plane of polarisation is rotated when polarised light passes through the substance, if liquid, or a solution of the substance. Substances are described as *dextro*-rotatory or *laevo*-rotatory according to whether the plane of polarisation is rotated clockwise or anticlockwise, respectively, as determined by viewing towards the light source. *Dextro*-rotation is designated (+) and *laevo*-rotation is designated (−).

The *optical rotation*, unless otherwise specified, is measured at the wavelength of the D line of sodium ($\lambda = 589.3$ nm) at 25°, on a layer 1 dm thick. It is expressed in degrees.

The *specific optical rotation* $[\alpha]_D^{25}$ of a liquid substance is the angle of rotation α of the plane of polarisation at the wavelength of the D line of sodium ($\lambda = 589.3$ nm) measured at 25°, calculated with reference to a 1.0 dm thick layer of the liquid, and divided by the specific gravity.

The *specific optical rotation* $[\alpha]_D^{25}$ of a solid substance is the angle of rotation α of the plane of polarisation at the wavelength of the D line of sodium measured at 25° and calculated with reference to a layer 1.0 dm thick of a solution containing 1 g of the substance per ml. The specific optical rotation of a solid is always expressed with reference to a given solvent.

Apparatus

A commercial instrument constructed for use with a sodium lamp and capable of giving readings to the nearest 0.02° is suitable for most purposes. For certain applications, the use of a photo-electric polarimeter capable of taking measurements at the specified wavelengths may be necessary.

The accuracy and precision of optical rotation measurements can be increased if the following precautions are taken:

(a) The instrument must be in a good condition. Optical elements must be very clean and in exact alignment. The match point should be close to the normal zero mark.

(b) The light source must be properly aligned with respect to the optical bench. It should be supplemented by a filtering system capable of isolating the D line from sodium light.

(c) Specific attention should be paid to temperature control of the solution and of the polarimeter.

(d) Differences between the initial readings or between observed and corrected optical rotation calculated as either specific optical rotation or optical rotation should not be more than one fourth of the range specified in the monograph for the substance.

(e) Polarimeter tubes should be filled in such a way as to avoid air bubbles. Particular care is necessary for semi-micro or micro tubes.

(f) For tubes with removable end-plates fitted with gaskets and caps, tighten the end-plates only enough to ensure a leak-proof seal between the end-plate and the body of the tube.

(g) For substances with low rotatory power, the end-plates should be loosened and tightened again after each reading, in the measurement of both the rotation and the zero point.

(h) Liquids and solutions of solids must be clear.

Calibration

The apparatus may be checked by using a solution of previously dried sucrose and measuring the optical rotation in a 2-dm tube at 25° and using the concentrations indicated below:

Concentration (g/100 ml)	Angle of Rotation (+), at 25°
10.0	13.33
20.0	26.61
30.0	39.86
40.0	53.06
50.0	66.23

Method

For solids : Weigh accurately a suitable quantity of the substance being examined, to give a solution of the

strength specified in the monograph, and transfer to a volumetric flask by means of *water*, or other solvent if specified. If a solvent is used, reserve a portion of it for the blank determination. Unless otherwise specified, adjust the contents of the flask to 25° by suspending the flask in a constant-temperature bath. Make up to volume with the solvent at 25° and mix well. Transfer the solution to the polarimeter tube within 30 minutes from the time the substance was dissolved and during this time interval maintain the solution at 25°.

Determine the zero point of the polarimeter and then make five readings of the observed rotation of the test solution at 25°. Take an equal number of readings in the same tube with the solvent in place of the test solution. The zero correction is the average of the blank readings, and is subtracted from the average observed rotation if the two figures are of the same sign or added if they are opposite in sign, to give the corrected observed rotation.

For liquids : Unless otherwise specified, adjust the temperature of the substance being examined to 25°, transfer to a polarimeter tube and proceed as described **For solids**, beginning at the words "Determine the zero point....".

Calculation

Calculate the specific optical rotation using the following formulae, *dextro*-rotation and *laevo*-rotation being designated by (+) and (−) respectively:

$$\text{For liquids } [\alpha]_D^{25} = \frac{\alpha}{l d_{25}^{25}}$$

$$\text{For solids } [\alpha]_D^{25} = \frac{100\ \alpha}{l c}$$

where

α = corrected observed rotation, in degrees, at 25°

D = D line of sodium light (λ = 589.3 nm)

l = length of the polarimeter tube in dm

d_{25}^{25} = specific gravity of the liquid or solution at 25°

c = concentration of the substance in per cent w/v

NOTE – The requirements for optical rotation and specific optical rotation in the Pharmacopoeia apply to the dried, anhydrous, or solvent-free material in all those monographs in which standards for loss on drying, water, or solvent content are given. In calculating the result the loss on drying, water, or solvent content determined by the method specified in the monograph is used.

5.13 POWDER FINENESS

The degree of coarseness or fineness of a powder is expressed by reference to the nominal mesh aperture size of the sieves used for measuring the size of the powders. For practical reasons, the use of sieves, Appendix 1.2, for measuring powder fineness for most pharmaceutical purposes, is convenient but devices other than sieves must be employed for the measurement of particles less than 100 μm in nominal size.

The following terms are used in the description of powders:

Coarse powder : A powder all the particles of which pass through a *sieve* with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a *sieve* with a nominal mesh aperture of 355 μm.

Moderately coarse powder : A powder all the particles of which pass through a *sieve* with a nominal mesh aperture of 710 μm and not more than 40.0 per cent through a *sieve* with a nominal mesh aperture of 250 μm.

Moderately fine powder : A powder all the particles of which pass through a *sieve* with a nominal mesh aperture of 355 μm and not more than 40.0 per cent through a *sieve* with a nominal mesh aperture of 180 μm.

Fine powder : A powder all the particles of which pass through a *sieve* with a nominal mesh aperture of 180 μm.

Very fine powder : A powder all the particles of which pass through a *sieve* with a nominal mesh aperture of 125 μm.

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder shall pass through a *sieve* of which the nominal mesh aperture, in μm, is equal to that number.

When a batch of a vegetable drug is being ground and sifted, no portion of the drug shall be rejected, but it is permissible, except in the case of assays, to withhold the final tailings, if an approximately equal amount of tailings from a preceding batch of the same drug has been added before grinding.

Sieves

Sieves for testing powder fineness comply with the requirements stated under *sieves*, Appendix 1.2.

Method

(1) For coarse and moderately coarse powders : Place 25 to 100 g of the powder being examined, upon the appropriate sieve having a close-fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction and vertically by tapping on a hard surface for not less than twenty minutes or until sifting is practically complete. Weigh accurately the amount remaining on the sieve and in the receiving pan.

(2) For fine and very fine powder : Proceed as described under **Coarse and moderately coarse powders**, except that the test sample should not exceed 25 g and except that the sieve is to be shaken for not less than thirty minutes, or until sifting is practically complete.

With oily or other powders which tend to clog the openings, carefully brush the screen at intervals during sifting. Break up any lumps that may form. A mechanical sieve shaker which reproduces the circular and tapping motion given to sieves in hand sifting but has a uniform mechanical action may be employed.

NOTE – Avoid prolonged shaking that would result in increasing the fineness of the powder during the testing.

5.14 REFRACTIVE INDEX

The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25° (± 0.5) with reference to the wavelength of the D line of sodium ($\lambda = 589.3$ nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe refractometer is convenient for most measurements of refractive index but other refractometers of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light. To achieve accuracy, the apparatus should be calibrated against distilled *water* which has a refractive index of 1.3325 at 25° or against the reference liquids given in the following Table:

TABLE

Reference Liquid	n_D^{20*}	Temperature Coefficient $\Delta n/\Delta t$
Carbon tetrachloride	1.4603	−0.00057
Toluene	1.4969	−0.00056
α-Methylnaphthalene	1.6176	−0.00048

*Refractive index value for the D line of sodium, measured at 20°

The cleanliness of the instrument should be checked frequently by determining the refractive index of distilled *water* which at 25° is 1.3325.

5.15 SPECTROPHOTOMETRY

Absorption Spectrophotometry is the measurement of the absorption of electromagnetic radiation of definite and narrow wavelength range, by molecules and atoms of a chemical substance. Techniques frequently employed in pharmaceutical analysis include ultra-violet, visible, infra-red and atomic absorption spectroscopy.

Fluorescence spectrophotometry is the measurement of the emission of light from a chemical substance while it is being exposed to ultra-violet, visible, or other electromagnetic radiation.

The wavelength range available for these measurements extends from the short wavelengths of the ultra-violet through the infra-red. For convenience of reference, this spectral range is roughly divided into the ultra-violet (185 nm to 380 nm), the visible (380 nm to 780 nm), the near infra-red(780 nm to 3000 nm) and the infra-red (3 μm to 40 μm).

For many pharmaceutical substances, measurements can be made in the ultra-violet and visible regions of the spectrum with greater accuracy and sensitivity than in the infra-red. When solutions are observed in 1-cm cells, concentrations of about 10 μg of the substance per ml will produce *extinctions* (absorbances) of 0.2 to 0.8 in the ultra-violet and visible region. In the infra-red and near infra-red regions, concentrations of 1 to 10 mg or upto 100 mg per ml, respectively, may be needed to produce sufficient absorption; for these spectral ranges, cell lengths of 0.01 mm and upwards upto 3 mm are commonly used.

The ultra-violet and visible spectra of substances generally do not have a high degree of specificity. Nevertheless, they are suitable for quantitative assays and for many substances they are useful as additional means of identification.

The infra-red absorption spectrum is unique for any given chemical compound with the exception of optical isomers, which have identical spectra. However, polymorphism may sometimes show differences in the infra-red spectrum of a given compound in the solid state. Because of the large numbers of maxima in an infra-red absorption spectrum, it is sometimes possible to measure quantitatively the individual components of a mixture of known qualitative composition without prior separation.

A. Ultra-violet and Visible Spectrophotometry

When radiation is passed through a layer of a solution containing an absorbing substance, part of the radiation is absorbed; the intensity of the radiation emerging from the solution is less than the intensity of the radiation entering it. The magnitude of the absorption is expressed in terms of the extinction, E which is defined by the expression:

$$E = \log_{10}(I_0/I)$$

where I_0 is the intensity of the radiation passing into the absorbing layer and I is the intensity of the radiation passing out of it.

The *extinction* depends on the concentration of the absorbing substance in the solution and the thickness of the absorbing layer taken for measurement. For convenience of reference and for ease in calculations, the

extinction of a 1-cm layer of a 1 per cent w/v solution is adopted in this Pharmacopoeia for several substances and is evaluated by the expression:

$$E\ (1\ \text{per cent, 1-cm}) = E/cl$$

where c is the concentration of the absorbing substance expressed as a percentage w/v and l is the thickness of the absorbing layer in cm. The value of E(1 per cent, 1-cm) at a particular wavelength in a given solvent is a property of the absorbing substance. This property is utilised for identifying substances and also in assays where solutions can be obtained which are free from interfering materials. In other cases, the pharmacopoeial tests and assays call for comparison against *Reference Substances (R.S.)* to ensure measurement under conditions identical for the substance under examination and the reference substance. Where a test or an assay prescribes the use of a *Reference Substance* the spectrophotometric measurements are made first with the solution prepared from the *Reference Substance* by the directions given in the individual monograph and then with the corresponding solution prepared from the substance under examination. The second measurement should be carried out immediately after the first, using the same cell and the same instrumental conditions.

1. Spectrophotometers : Spectrophotometers suitable for measuring in the ultra-violet and visible range of the spectrum consist of an optical system capable of producing monochromatic light in the range 190 to 780 nm and a device suitable for measuring the extinction.

The two empty cells used for the solution being examined and the reference liquid must have the same spectral characteristics. Where double-beam recording instruments are used the solvent cell is placed in the reference beam.

2. Control of wavelengths : Verify the wavelength scale using the absorption maxima of *holmium perchlorate solution*, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapour are shown below. The permitted tolerance is ± 1 nm for the range 200 to 400 nm and ± 3 nm for the range 400 to 600 nm.

241.15 nm (Ho)	404.66 nm (Hg)
253.70 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.00 nm (Dβ)
302.25 nm (Hg)	486.10 nm (Hβ)
313.16 nm (Hg)	536.30 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.50 nm (Ho)	576.96 nm (Hg)
365.48 nm (Hg)	579.07 nm (Hg)

3. Control of extinction : Check the *extinction* using *potassium dichromate solution UV* at the wavelengths indicated in the following Table.

TABLE

Wavelength (nm)	E(1 per cent, 1-cm)	Maximum Tolerance
235	124.5	122.9 to 126.2
257	144.0	142.4 to 145.7
313	48.6	47.0 to 50.3
350	106.6	104.9 to 108.2

4. Limit of stray light : Stray light may be detected at a given wavelength with suitable filters or solutions; for example the *extinction* of a 1.2 per cent w/v solution of *potassium chloride* at a path-length of 1 cm should be greater than 2 at 200 nm when compared with *water* as reference liquid.

5. Spectral slit width : When measuring the extinction at an absorption maximum the spectral slit width must be small compared with the half-width of the extinction band, or erroneously low extinction will be measured. Particular care is needed for certain substances and the instrumental slit width used should always be such that further reduction does not result in an increased extinction reading.

6. Cells : The extinction of the cells intended to contain the solution to be examined and the reference liquid when filled with the same solvent, should be identical. If this is not the case, an appropriate correction must be applied. The tolerance on the path length of the cells used is ± 0.005 cm. Cells should be cleaned and handled with great care.

7. Preparation of sample : The substance being examined is generally dissolved in a solvent. Care should be taken to use solvents free from contaminants absorbing in the spectral region being used. In measuring the extinction of a solution at a given wavelength, the extinction of the solvent cell and its contents shall not exceed 0.4 and is preferably less than 0.2 when measured with reference to air at the same wavelength. The solvent in the solvent cell shall be of the same batch as that used to prepare the solution and must be free from fluorescence at the wavelength of measurement. *Alcohol, ethyl alcohol, methyl alcohol* and *cyclohexane UV* used as solvents shall have an *extinction*, measured in a 1-cm cell at 240 nm with reference to *water*, not exceeding 0.10.

8. Measurement of extinction : Unless otherwise prescribed, measure the *extinction*, E, at the prescribed wavelength using a path-length of 1 cm at 25° ± 1°. Measurements are normally carried out with reference to the solvent used to prepare the solution being examined, unless otherwise indicated in the individual monograph.

When the extinction is being measured for a quantitative determination, for example, an assay or a limit test, a

manually-scanning instrument is used. In tests for identification, it is more convenient to use a recording instrument and the concentration of the solution and the path-length are specified accordingly. If these conditions are not appropriate for a particular instrument, the thickness and not the concentration of the solution should be varied. A statement in an assay or test of the wavelength at which maximum absorption occurs implies the maximum occurring either precisely at or in the vicinity of the given wavelength.

Quantitative determinations are usually carried out at wavelength above 235 nm. If the measurements are to be made at a wavelength in the range 190–210 nm, special precautions should be observed, for example, purging the cell compartment with nitrogen, use of solvents of special spectroscopic quality and using cells that are transparent in this region.

The requirements for light absorption in the Pharmacopoeia apply to the dried, anhydrous, or solvent-free material in all those monographs in which standards for loss on drying, water or solvent content are given. In calculating the result the loss on drying, water, or solvent content determined by the method specified in the monograph is used.

B. Infra-red Spectrophotometry

1. Spectrophotometers : The apparatus for recording the spectra in the infra-red region consists of an optical system capable of providing monochromatic light in the region of 4000 to 670 cm^{-1} (about 2.5 to 15 um) and a means of measuring the quotient of the intensity of the transmitted light and the incident light. For recording infra-red spectra, the instrument should comply with the following test for resolution.

Record the spectrum of a polystyrene film 0.05 mm in thickness. The depth of the trough from the maximum at about 2851 cm^{-1} (3.51 μm) to the minimum at about 2870 cm^{-1} (3.48 μm) should be greater than 6 per cent transmittance for prism instruments and 18 per cent transmittance for grating instruments, and that from the maximum at about 1583 cm^{-1} (6.32 μm) to the minimum at about 1589 cm^{-1} (6.29 μm) should be greater than 6 per cent transmittance for prism instruments and 12 per cent transmittance for grating instruments.

2. Preparation of sample : A sample of the substance being examined may be prepared in one of the following ways:

Liquids—Examine a liquid as a thin film held between two plate or in a cell of suitable path-length constructed of material transparent to infra-red rediation in the region to be examined.

Liquids or solids prepared as solutions—Prepare a solution in a suitable solvent, and use a concentration and path-length to give a satisfactory spectrum over a sufficiently wide wavelength range. Absorption due to the solvent should be compensated for by placing in the reference beam a similar cell containing the solvent used; it should be noted that absorption bands due to the substance under examination that coincide with strong solvent absorption will not be recorded. Suitable concentrations of the solute will vary with the substance being examined but typical concentrations are 1 to 10 per cent at 0.5 to 0.1 mm path-length.

Solids—Examine a solid after dispersion in a suitable liquid (mull) or solid (halide disc).

Mulls—Triturate 1 to 5 mg of the substance with the minimum amount of *liquid paraffin* or other suitable liquid to give a smooth creamy paste. Compress a portion of the mull between two suitable plates.

Discs—Triturate about 1 mg of the substance with approximately 300 mg of dry, finely powdered *potassium bromide IR* or *potassium chloride IR*, as directed. These quantities are usually suitable for a disc 13 mm in diameter. Grind the mixture thoroughly, spread it uniformly in a suitable die and compress under vacuum at a pressure of about 10 t cm^{-2}. Commercial dies are available and the maker's instructions should be followed. Mount the resultant disc in a suitable holder in the spectrophotometer. Several factors, such as inadequate or excessive grinding, moisture or other impurities in the halide carrier, may give rise to unsatisfactory discs. Unless its preparation presents particular difficulties a disc should be rejected if visual inspection shows lack of uniformity or if the transmittance at about 2000 cm^{-1} (5 μm) in the absence of a specific absorption band is less than 75 per cent without compensation. If the other ingredients of tablets, injections, or other dosage forms are not completely removed from the substance being examined, they may contribute to the spectrum.

3. Identification by reference substances : Prepare the substance being examined and the appropriate Reference Substance by the same procedure and record the spectrum of each from about 3800 to 625 cm^{-1} (2.5 to 15 μm) using the same instrumental conditions as were used to demonstrate compliance with the requirement for resolution.

NOTE – Pretreatment of the sample may sometimes be necessary with substances known to exhibit polymorphism which could lead to differences between the spectra of the substance and of the Reference Substance. Ingredients of tablets, injections or other dosage forms may contribute to the spectrum if they are not completely removed from the substance under examination.

C. Fluorescence Spectrophotometry

This procedure uses the measurement of the intensity of the fluorescent light emitted by the substance being examined in relation to that emitted by a given reference standard.

Apparatus : Operate a spectrophotofluorimeter in accordance with the manufacturing instructions.

Method : Dissolve the substance being examined in the solvent prescribed in the monograph, transfer the solution to the cell of the spectrophotofluorimeter and illuminate it with an excitant light beam of the nominal wavelength prescribed in the monograph and as nearly monochromatic as possible. Measure the intensity of the emitted light at the specified wavelength of the fluorescence.

For quantitative analysis introduce into the apparatus the solvent used to dissolve the substance being examined and set the instrument to zero. Introduce the prescribed standard solution and adjust the sensitivity of the instrument so that the reading is close to the maximum. If the second adjustment is made by altering the width of the slits, a new zero setting must be made and the intensity of the standard must be measured again. Finally introduce the solution of the substance being examined, record the intensity of fluorescence, and calculate its concentration.

If the intensity of the fluorescence is not directly proportional to the concentration, the measurement may be effected using a calibration curve. In some cases, measurement can be made with reference to a fixed standard such as a solution of stated concentration of quinine in *0.1N sulphuric acid* or of fluorescein in *0.1N sodium hydroxide*. In such cases, the concentration of the substance being examined must be determined using a calibration curve previously prepared under the same conditions.

5.16 ATOMIC SPECTROPHOTOMETRY

A. Flame Photometry

The following methods are based on the measurement of intensity of spectral-lines omitted by elements such as sodium, potassium, calcium, etc. The substance containing the element is dissolved in an appropriate solvent (usually water) and subjected to excitation in a flame of appropriate temperature and composition.

Apparatus : Several instruments of suitable selectivity are available. The manufacturer s instructions for the operation of the flame photometer should be followed.

Methods : Unless otherwise directed one or the other of the following methods may be used. In Method A, measurements are made by comparison of sample solutions with solutions containing a known amount of the element being analysed. Method B is suitable for samples which contain very small quantities of the element to be analysed or where there is interference from other elements.

Method A – Prepare a series of standard solutions containing the element to be determined in increasing concentration within the concentration range recommended for the particular instrument used. Choose the appropriate filters or adjust the monochromator to select the wavelength prescribed in the monograph. Spray *water* into the flame and adjust the galvanometer reading to zero. Spray the most concentrated standard solution into the flame and adjust the sensitivity so that a full-scale deflection of the galvanometer is recorded. Again spray *water* into the flame and when the galvanometer reading is constant readjust it to zero. Spray each standard solution into the flame three times, recording the steady galvanometer readings obtained and washing the apparatus thoroughly with *water* after each spraying. Prepare a calibration curve by plotting the mean of each group of three readings against the concentration. Prepare the solution of the substance to be examined as prescribed in the monograph, and adjust the strength, if necessary, to bring it into the range of concentration recommended for the instrument used. Spray the solution into the flame three times, recording the galvanometer readings and washing the apparatus through with *water* after each spraying. Using the mean of the galvanometer readings, determine the concentration of the element being examined from the calibration curve. To confirm the concentration thus obtained, repeat the operations with a standard solution of the same concentration as that of the solution being examined.

Method B – Place in each of not fewer than three similar graduated flasks equal volumes of the solution to be examined, prepared as prescribed in the monograph. Add to all but one of the flasks a measured amount of the prescribed standard solution to produce a series of solutions containing regularly increasing amounts of the element to be determined. Dilute the contents of each flask to the required volume with *water*.

Prepare the flame photometer in the manner described under Method A, using *water* for the adjustment to zero and the solution with the largest amount of added element to adjust the sensitivity. Examine each solution three times and plot on the co-ordinate of a graph, whose origin represents zero concentration of the added element, the calculated final concentration of each solution after taking account of the dilution; on the ordinate of the graph plot the mean of the corresponding galvanometer readings. Extrapolate the straight line joining the points until it meets the concentration axis at a point on the negative side. The distance between this point and the origin represents the concentration of the element in the solution to be examined.

B. Atomic Absorption Spectrophotometry

This technique is based on the fact that when atoms, ions or ion complexes of an element are atomised to the ground state in a flame, they absorb light at the characteristic wavelength of that element. If the absorption process takes place in the flame under reproducible conditions, the absorbance is proportional to the number of absorbing atoms.

Apparatus : An atomic absorption spectrophotometer consists of an emission source that provides the characteristic spectral line of the element such as a hollow-cathode

discharge lamp, a monochromator to select the required resonance line, a nebuliser-burner system for introducing the sample solution into a flame and a detector system.

Since the radiation to be absorbed by the element in the test solution is usually of the same wavelength as that of its emission line, the element in the hollow-cathode lamp is the same as the element to be determined and usually a different lamp is used for each element. The nebuliser-burner system introduces the test solution into a flame, which is usually provided by an acetylene or air-hydrogen.

The manufacturer's instructions for the operation of the instrument should be followed.

Methods : Unless otherwise directed in the individual monograph one or the other of the following methods may be used. In Method A the measurement is made by comparison with solutions containing a known amount of the element to be determined by means of a calibration graph and in Method B comparison is made by means of progressive addition of the reference solution of the element to be determined.

Method A — Prepare not fewer than three standard solutions of the element to be determined, covering the concentration range recommended by the manufacturers for the instrument and element used. Any reagent used in the preparation of the solution of the substance being examined should be added to the standard solutions in the same concentration. After calibrating the instrument as directed above, introduce each standard solution into the flame three times, and record the steady reading, washing the apparatus through with *water* after each introduction. Prepare a calibration curve by plotting the mean of each group of three readings against the concentration. Prepare a solution of the substance being examined as directed in the monograph, adjusting the concentration to fall within the concentration range of the standard solutions. Introduce the solution into the flame, record the reading and wash the apparatus through with *water*. Repeat the sequence twice and, using the mean of the three readings, determine the concentration of the element from the calibration curve.

Method B — Place in each of not fewer than three similar volumetric flasks equal volumes of the solution of the substance being examined, prepared as directed in the monograph. Add to all but one of these flasks a measured amount of the specified standard solution to produce a series of solutions containing steadily increasing amounts of the element being determined. Dilute the contents of each flask to the required volume with *water*.

After calibrating the spectrometer as directed above, record the reading of each solution three times. Plot the mean of the readings against concentration of a graph the axes of which intersect at zero added element and zero reading. Extrapolate the straight line joining the points until it meets the extrapolated concentration axis. The distance between this point and the intersection of the axis represents the concentration of the element being determined in the solution of the substance being examined.

NOTE — For the purposes of this Appendix water refers to de-ionised water obtained from an ion-exchange resin (Amberlite MB-1 is suitable), distilled immediately before use.

5.17 DETERMINATION OF SPECIFIC SURFACE AREA

The surface area of powders is determined by an equipment such as a subsieve sizer which is designed for measurement of average particle sizes in the range of 0.2 to 50 microns. The instrument operates on the air-permeability principle which employs the fact that a current of air flows more readily through a bed of coarse powder than through an otherwise equal bed of fine powder that is equal in shape of bed, apparent volume, and percentage of voids, but by reason of difference in general coarseness of material, measurements of average particle sizes are obtained.

The apparatus consists of a sample tube containing the packed powder sample with one end connected to an air pump and pressure regulator and the other end attached to a calibrated manometer containing a suitable liquid of low viscosity and negligible vapour pressure. The air pump builds up air pressure to a constant head in the pressure regulator. Under this pressure head, air is conducted to the packed powder in the sample tube and the flow of air through this packed powder bed is measured by the manometer, in which the level of the fluid indicates the average diameter of the powder particles. Commercially available equipment is standardised to eliminate mathematical computation and average particle diameters can be read from the calculator charts provided with the equipment. The relationship between average particle diameter and specific surface area is given by the expression:

$$SSA = \frac{6 \times 10^4}{d \times p}$$

where

SSA = Specific surface area in cm^2 per g of material

d = Average diameter in microns (obtained from the calculator chart)

p = True density of material from which the powder was made in g per cm^3.

The equipment manufacturer's instructions for setting up the apparatus, preparing the compacted powder bed, operating the instrument and calculating the results must be followed.

Method for Bephenium Hydroxynaphthoate — Prepare a compact 2.54 cm in diameter and 1.00 cm in height, with a porosity within the range 0.475 to 0.525 in the sample tube and measure the permeability of the com-

pact. Calculate the specific surface area assuming the true density of the powder to be 1.298 per cm^3.

Method for Griseofulvin – Weigh 1.819 g ± 0.001 g and transfer to the sample tube. Compact the powder with moderate pressure and measure the permeability of the compact. Calculate the specific surface area (SSA) assuming the true density of the powder to be 1.455 g per cm^3. Divide the result by 10^4 to give the specific surface area in square metres per gram.

5.18 DETERMINATION OF VISCOSITY

Viscosity is a property of a liquid which is closely related to the resistance to flow.

In C.G.S. system, the dynamic viscosity (n) of a liquid is the tangential force in dynes per square centimetre exerted in either of the two parallel planes placed, 1 centimetre apart when the space between them is filled with the fluid and one of the planes is moving in its own plane with a velocity of 1 centimetre per second relative to the other. The unit of dynamic viscosity is the poise (abbreviated P). The centipoise (abbreviated cP) is 1/100th of one poise.

While on the absolute scale, viscosity is measured in poise or centipoise, it is most convenient to use the kinematic scale in which the Units are stokes (abbreviated St) and centistokes (abbreviated cSt). The centistoke is 1/100 th of one stoke. The kinematic viscosity of a liquid is equal to the quotient of the dynamic viscosity and the density of the liquid at the same temperature, i.e.,

$$\text{Kinematic Viscosity} = \frac{\text{Dynamic Viscosity}}{\text{Density}}$$

The determination of viscosity of newtonian liquids is carried out be means of a capillary viscometer, unless otherwise specified; Methods A and B described below are recommended. For non-newtonian liquids Method C using the rotating viscometer may be used.

For measurement of viscosity, the temperature of the substance being measured must be accurately controlled, since small temperature changes may lead to marked changes in viscosity. For usual pharmaceutical purposes, the temperature should be maintained to within ±0.1°.

Method A (Using Ostwald-type Viscometer)

Apparatus : The apparatus consists of a glass U-tube viscometer (*see* Fig. 5) made of clear borosilicate glass and constructed in accordance with the dimensions shown in the figure in Table 1.

Method : Fill the viscometer (previously washed and completely dried) with the liquid being examined through tube L to slightly above the mark G, using a long pipette to minimise wetting the tube above the mark. Place the tube vertically in a water-bath maintained at the temperature indicated in the monograph and allow to stand for not less than 30 minutes to allow the temperature to reach equilibrium. Adjust the volume of the liquid so that the bottom of the meniscus settles at the mark G. Suck or blow the liquid to a point about 5 mm above the

TABLE 1

Size No.	Kinematic Viscosity Range (Centistokes)	Inside Diameter of Tube R (±2%)	Outside Diameter of Tubes*		Volume Bulb C (ml) (±5%)	Vertical Distance F to G (mm)	Outside Diameter of Bulbs A and C (mm)
			L and P (mm)	N (mm)			
A	0.9** to 3	0.50	8 to 9	6 to 7	5.0	91 ± 4	21 to 23
B	2.0 to 10	0.71	8 to 9	6 to 7	5.0	87 ± 4	21 to 23
C	6 to 30	0.88	8 to 9	6 to 7	5.0	83 ± 4	21 to 23
D	20 to 100	1.40	9 to 10	7 to 8	10.0	78 ± 4	25 to 27
E	60 to 300	2.00	9 to 10	7 to 8	10.0	73 ± 4	25 to 27
F	200 to 1000	2.50	9 to 10	7 to 8	10.0	70 ± 4	25 to 27
G	600 to 3000	4.00	10 to 11	9 to 10	20.0	60 ± 3	3 to 35
H	2000 to 10,000	6.10	10 to 11	9 to 10	20.0	50 ± 3	32 to 35

* Use 1 to 1.25 mm wall tubing for N, P and L
** 300s minimum flow time; 200s minimum flow time for all other sizes

mark E. After releasing pressure or suction, measure the time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F.

Calculate the kinematic viscosity in centistokes (V) from the expression

$$v = Ct,$$

where

t = time in seconds for the meniscus to fall from E to F

C = the constant of the viscometer, determined by observations on a liquid of known viscosity.

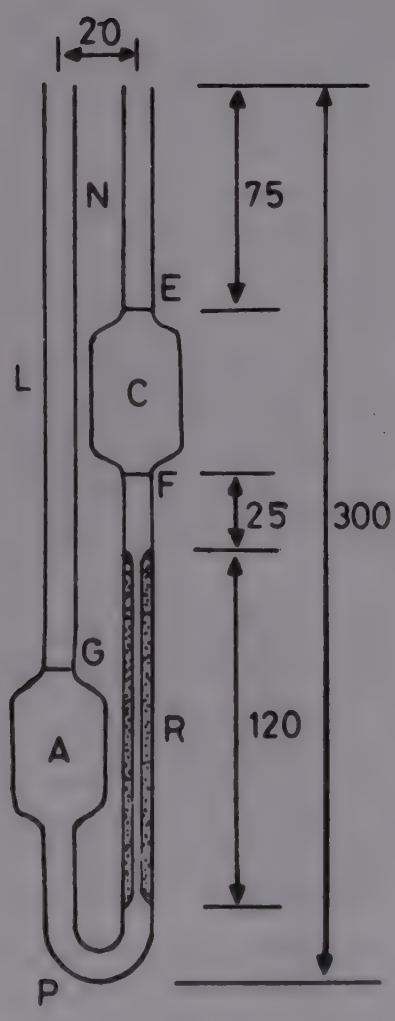
NOTE – For Dextran Injections, the viscosity ratio is calculated by dividing the time taken with the liquid under examination by the time taken by saline solution for the meniscus to fall from E to F.

Method B: (Using the Suspended-level Viscometer)

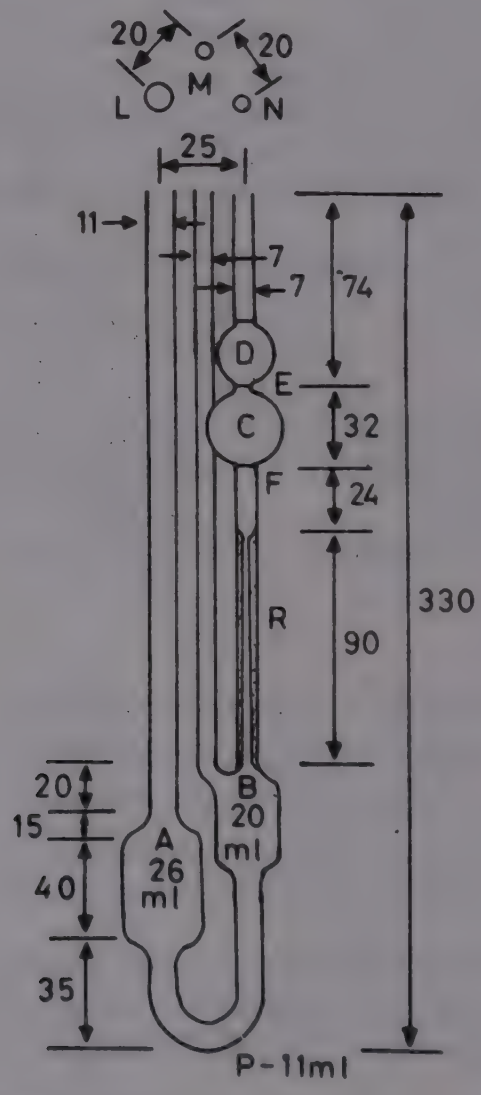
Apparatus : The apparatus consists of a glass suspended-level viscometer (*see* Fig. 6) made of clear borosilicate glass and constructed in accordance with the dimensions shown in Table 2.

Method : Fill the viscometer through tube L with a sufficient quantity of the liquid being examined to ensure that bulb C is satisfactorily filled without blocking the ventilation tube M. After the tube has been placed vertically in the bath and has attained the specified temperature, close tube M and apply suction to tube N until the liquid reaches a level about 5 mm above mark E. Hold the liquid at this level by closing tube N and open tube M. When the liquid is clear of the capillary end of tube N and the lower end of tube M, open tube N. Measure the time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F.

If the end of tube M becomes blocked by the liquid at



Ostwald-type Viscometer
(Fig.5)



Suspended-level Viscometer
(Fig.6)

(Dimensions in mm unless otherwise stated; tolerance are ± 10% or ± 10 mm, whichever is the less

TABLE 2

Size No.	Kinematic Viscosity Range (Centistokes)	Volume Bulb C (ml) (±5%)	Inside Diameter of Tube N (mm)	Inside Diameter of Tube R (mm) (±2%)
1	3.5* to 10	0.64	5.6	2.8 to 3.2
1A	5 to 30	0.84	5.6	2.8 to 3.2
2	20 to 100	1.15	5.6	2.8 to 3.2
2A	60 to 300	1.51	5.6	2.8 to 3.2
3	200 to 1100	2.06	5.6	3.7 to 4.3
3A	600 to 3000	2.74	5.6	4.6 to 5.4
4	2000 to 10,000	3.70	5.6	4.6 to 5.4
4A	6000 to 30,000	4.97	5.6	5.6 to 6.4
5	20,000 to 100,000	6.76	5.6	6.8 to 7.5

*350 minimum flow time; 200 minimum flow time for all other sizes

any time while the flow time is being measured, the determination must be repeated.

Calculate the kinematic viscosity in centistokes (V) from the equation:

$$v = Ct,$$

where

t = time in seconds for the meniscus to fall from E to F

C = the constant of the viscometer, determined by observations on a liquid of known viscosity.

Method C : (Using the Rotating Viscometer)

The rotating viscometer measures the shearing forces in a liquid medium placed between two coaxial cylinders one of which is driven by a motor and the other is caused to revolve by the rotation of the first. Under these conditions, the viscosity becomes a measurement of the angle of deflection of the cylinder caused to revolve, expressed in newton metres.

Method—Operate the Rotating Viscometer in accordance with the manufacturer's instructions and carry out the determination of viscosity of the liquid being examined, at the temperature and angular velocity or shear rate specified in the individual monograph.

Calculate the dynamic viscosity (n) in centipoises.

5.19 WEIGHT PER MILLILITRE AND SPECIFIC GRAVITY

Weight per Millilitre

The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25°, unless otherwise specified.

Method : Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled *water* at 25° and weighing the contents. Assuming that the weight of 1 ml of *water* at 25° when weighed in air of density 0.0012 g per ml, is 0.99602 g, calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20° and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per millilitre by dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Specific Gravity

The specific gravity of a liquid is the weight of a given volume of the liquid at 25° (unless otherwise specified) compared with the weight of an equal volume of *water* at the same temperature, all weighings being taken in air.

Method : Proceed as described under **Wt. per ml.** Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of *water* contained, both determined at 25° unless otherwise directed in the individual monograph.

6. General Information

6.1 CLEANING OF GLASSWARE

Glassware such as flasks, beakers, pipettes, burettes, etc. employed in conducting many of the pharmacopoeial assays should be very clean. This is particularly important for certain microbiological assays and where small volumes of liquids are measured.

A very effective cleaning fluid is the chromic acid mixture consisting of 200 g of sodium dichromate dissolved in about 100 ml of water cooled in an icebath to which 1500 ml of sulphuric acid has been added slowly and with stirring. This mixture should be prepared in a hard, borosilicate glass beaker; safety goggles must be worn during the addition of the acid. It is extremely corrosive and hygroscopic and should be stored in closed glass-stoppered bottles. If a green colour develops, discharge the mixture in a sink with continuously flowing water.

Glass treated with chromic acid mixture should be subject to prolonged rinsing as glass tends to adsorb the chromic acid.

Chromic acid should not be used for cleaning calibrated containers used for optical measurements.

For general cleaning of glassware, synthetic detergent solutions or alkaline cleansing agents such as trisodium phosphate may be used, but these require prolonged rinsing.

All glassware should be finally rinsed with *purified water* before use.

6.2 METHODS OF STERILISATION

The choice of a method for attaining sterility in a product or article is governed by the nature of the product or article, the extent and type of any contamination present and in the case of a product the conditions under which it has been manufactured. Materials to be sterilised should be as free as possible from microbial contamination.

The sterilisation process requires vigilant supervision of equipment and of procedures for operation, maintenance and calibration of such equipment. Failure to follow a process meticulously involves the risk of a non-sterile or deteriorated product. The effectiveness of any sterilising process must be adequately validated before it is applied in practice.

The establishment, validation, and monitoring of the sterilisation process should be done by incorporating appropriate physical, chemical and microbiological measurements to ensure that the desired effect on the product (including its final container or package, where applicable) is achieved. Additionally, the microbial burden on the product should be periodically monitored prior to sterilisation to ensure that contamination is at levels not greater than the ones established on validation of the process.

The following methods of sterilisation are recommended for the articles and products of the Pharmacopoeia:

1. Steam Sterilisation

This process involves heating in an autoclave employing saturated steam under pressure. The following combinations of temperature and time are normally employed for sterilising by heating in an autoclave.

Holding temperature	Minimum holding time in minutes
115° to 118°	30
121° to 124°	15
126° to 129°	10
134° to 138°	3

Other combinations of time and temperature may be used provided that they can be demonstrated to be effective.

The prescribed conditions are those to be maintained throughout the load, during the holding period of the sterilisation cycle.

A product which is to be sterilised by steam sterilisation is distributed into suitable containers, which are then sealed so as to exclude micro-organisms. It is then exposed to saturated steam for a time sufficient to ensure that the entire contents of each container are maintained for an effective combination of time and temperature to ensure sterility.

Small quantities of aqueous preparations may be satisfactorily sterilised with bench autoclaves but with larger quantities it would be necessary to standardise the conditions within the autoclave chamber. The temperature at which autoclaves are operated are measured by temperature-sensing devices located in the steam discharge line of the autoclaves. Adequate steps must be taken to ensure that all air in the chamber has been removed prior to initiating the sterilising cycle. The time the product is exposed to temperature (heat-up, holding and cooling time) varies with the nature of the product and the size of the containers being treated.

An indication of the actual conditions in the autoclave cannot be obtained only from a record of the temperature at the steam discharge line. It is important to check the distribution of heat in the sterilising chamber. For this purpose suitable devices are placed to register the temperatures attained at different horizontal and vertical planes in the chamber. The penetration of heat into the various parts of the load in the chamber should be monitored by inserting into representative containers recording- and temperature-sensitive elements as well as additional elements at the previously established coolest parts of the loaded chamber, other types of temperature indicators may be inserted at appropriate positions in the load but total reliance should not be placed on chemical indicators except when they suggest failure to attain sterilising conditions. A biological evaluation of the process may be done by including suitable heat-resistant spores in test containers in the load and subsequently testing these containers for sterility.

It is desirable that each sterilisation cycle be recorded on a temperature-time chart. Recording thermo-couples or thermometers should be periodically calibrated with certified mercury-glass thermometers.

When surgical dressings are sterilised by steam, the steam used should not contain more than 5 per cent of entrained moisture. Most dressings are conveniently sterilised by maintaining at a temperature of 134° to 138° for three minutes but other suitable combinations of temperature and time may be used.

2. Dry-heat Sterilisation

This process of sterilisation may be used for heat-stable non-aqueous products and powders. A preparation to be sterilised by dry heat is distributed in the final containers which are then either finally sealed or temporarily closed to exclude micro-organisms and then heated so as to ensure that the entire contents of each container are maintained at 160° for two hours, unless otherwise stated in the individual monograph, or at any other equally effective combination of temperature and time. Containers which have been temporarily closed during the sterilisation procedure are then finally sealed using aseptic technique so as to exclude micro-organisms.

Dry-heat sterilisation is usually carried out in a sterilising oven designed specifically for the purpose. The oven may be heated by gas or electricity and is normally provided with a fan or blower to circulate the heated air to assure adequate heat distribution throughout the chamber and with suitable temperature-recording devices.

Specific time and temperature schedules must be determined for each type of material treated, and when the nature of the material permits, a generous safety factor should be included in the exposure time to compensate for differences in the heat-up period and for other variables that may effect the cycle. The penetration of heat in the oven will depend on the size and nature of the units of material being sterilised. In general, individual units should be as small as possible and the steriliser should be loaded in such a manner as to permit free circulation of heated air throughout the chamber.

The effectiveness of any dry-heat sterilisation schedule should be determined under actual working conditions by means of thermo-couples or other suitable devices placed in representative locations within the load and, after a proper schedule has been determined, the chamber should be loaded in substantially the same manner for all subsequent cycles in which the particular material is to be treated. Suitable biological indications may be employed to demonstrate the effectiveness of the sterilisation cycle.

3. Sterilisation by Filtration

This process depends upon the physical removal of organisms by adsorption on a filter medium; it is used for the sterilisation of heat-sensitive solutions. A suitable filter medium yields a sterile and relatively particle-free filtrate and does not alter substantially the composition of the liquid passed through it.

In this process the filter, the receiving vessel, and any required accessories are sterilised by suitable means determined by the nature of the components in the system. The solution to be sterilised is then passed through the filter (usually made of cellulose derivatives, plastic, porous ceramic or of suitable sintered construction, or suitable combinations of these) and collected in the sterile receiver by the application of positive pressure to the non-sterile side of the system or application of vacuum to the sterile side. Care must be taken to avoid excessive positive or negative pressure, which may lead to failure of the sterilisation process. Prolonged filtration must also be avoided to prevent the growth of a contaminant through the filter medium and entry into the sterile solution. Appropriate measures should be taken to avoid loss of solute by adsorption on to the filter. After filtration the solution is aseptically distributed in previously-sterilised final containers which are then sealed so as to exclude micro-organisms.

Filter elements of porcelain or sintered material that are used repeatedly should be tested for cracks or leaks prior to each use. Fibre-shedding filters such as asbestos filters are to be avoided. If, however the use of such filters is unavoidable, a fibre-retaining filter must be used downstream of the asbestos filter. The effective pore size of the sterilising filters should be confirmed before use and the continued integrity of the filters confirmed after use.

The process of sterilisation by filtration must be validated by monitoring the microbial load in the solution to be filtered. For filters of a sintered or fritted construction a procedure such as the bubble-point procedure should be used and for membrane-type filters, a bubble-point procedure or diffusion rate test should be used, in accordance with the manufacturer's recommendations.

The integrity of any new or modified filtration system should be determined before it is placed in service.

4. Gas Sterilisation

This process involves exposure of materials to sterilising gases such as ethylene oxide. The process is difficult to control, the gas itself being toxic and when mixed in certain proportions with air, explosive. It must be ensured that the process is applied only to materials compatible with the sterilising gas.

The sterilising efficiency of ethylene oxide is related to the concentration of the gas, humidity, the time of exposure and the temperature. The material to be sterilised is exposed to a mixture of ethylene oxide with a suitable inert gas. After sterilisation adequate time should be allowed for the dispersal of residual ethylene oxide and other volatile residue. Products sterilised in this manner should be monitored to assure acceptable levels of residual gas and its degradation products.

The sterilisation cycle parameters must be determined carefully by the inclusion in each sterilisation cycle, bacteriological test pieces distributed throughout the load. These usually consist of aluminium foil on which has been dried at least one million viable and potentially recoverable spores of *Bacillus subtilis* var. *niger* (NCTC 10073). For each sterilisation cycle at least ten spore strips should be used.

The sterilisation cycle must be monitored by employing modern instrumentation for controlling the concentration of ethylene oxide, the temperature, the time and the moisture content in the sterilising chamber. As with the other sterilisation procedures, the microbial burden on the articles to be sterilised should be worked out, since this is an important factor which influences the effectiveness of the process.

5. Sterilisation by Ionising Radiation

Some materials can be sterilised by exposure of the product in its final container or package to ionising radiation in the form of Gamma radiation from a suitable radio isotonic source such as cobalt-60 or cesium-137 or of electrons energised by a suitable electron accelerator. A dose of 2.5 Mrads is generally accepted as adequate although other dosage levels may be employed. As with other sterilisation processes, the effectiveness of the radiation cycle should be confirmed by a series of experiments and by determination of the sterilisation microbial burdens and by the use of suitable dosimeters. Suitable biological indicators may also be used.

6. Sterilisation by Heating with a Bactericide

This process may be used for sterilising aqueous preparations which are unstable at the higher temperatures attained in the steam sterilisation processes. However, it has a lower margin of safety and should be used only when steam sterilisation is not applicable.

In this process the medicament is dissolved or suspended in a suitable solution of the following bactericides so as to achieve the concentration of bactericides given below:

For injections:

Chlorocresol	0.2 per cent w/v
Phenylmercuric acetate or Phenylmercuric nitrate	0.002 per cent w/v

For eye drops:

Benzalkonium chloride solution sufficient to give a final concentration of 0.01 per cent w/v of benzalkonium chloride

Phenylmercuric acetate or Phenylmercuric nitrate	0.002 per cent w/v
Thiomersal	0.01 per cent w/v
Chlorhexidine acetate	0.01 per cent w/v

The preparation is distributed in suitable container which are than sealed so as to exclude micro-organisms and heated for a sufficient time to ensure that the entire contents of each container are maintained at about 100° for thirty minutes.

The bactericide used must not interfere with the therapeutic efficacy of the product, or be incompatible with the product, its container or its closure.

Sterilisation by heating with a bactericide must not be used for

(1) solutions of medicaments for intrathecal, intracisternal or any other route of injection giving access to the cerebrospinal fluid; or

(2) for intracardiac or intraocular injections

6.3 WATER FOR PHARMACEUTICAL USE

Water is one of the most widely and abundantly used substances in pharmaceutical manufacturing. It is required for a variety of purposes ranging from manufacturing processes to the preparation of the final dosage forms. The quality of water therefore assumes considerable importance. Standards have been given in this Pharmacopoeia for the various types of water to be used in the manufacture of pharmaceuticals.

All forms of water covered by the relevant monographs in the Pharmacopoeia are prepared from drinking water which should conform to the regulations laid down by Public Health authorities. Drinking water may be used in some stages of manufacture of basic drug substances but not in the preparation of the final dosage forms or in the preparation of reagents or of test solutions.

The Pharmacopoeia has monographs for the following types of water:

1. Purified water : This substance represents water rendered suitable for pharmaceutical use by distillation, by treatment with ion-exchange materials, by reverse osmosis or by any other suitable process. It contains no

added substance and meets the requirements for chemical purity specified for it. However, it is liable to get contaminated by micro-organisms. It should therefore be monitored frequently for its bacteriological quality.

2. Water for injection : This substance is drinking water purified by distillation and it meets the purity require ments stated under Purified Water. It need not be sterile but it should comply with the *test for pyrogens*, Appendix 2.36, for which purpose it should be produced, stored and distributed in such a way that pyrogens are not produced by any micro-organisms, if present.

3. Sterile water for injection : This substance is *water for injection* which is sterilised within 12 hours after collection and distributed in sterile containers. It should be packaged only in single dose containers not larger than 1 litre. It contains no added substances.

7. Reagents and Solutions

7.1 BUFFER SOLUTIONS

A. Standard Buffer Solutions

Standard Buffer Solutions are solutions of standard pH. They are used for reference purposes in pH measurements and for carrying out many pharmacopoeial tests which require adjustments to or maintenance of a specified pH. They may be prepared by the methods described below: The preparation of special buffer solutions is described in the sections in which their use is specified as in Microbiological Assay of Antibiotics, or in the individual monographs where the use of such solutions is indicated.

The reagents required for the preparation of standard buffer solutions are described in the section, General Reagents, Appendix 7.4. All the crystalline reagents except boric acid should be dried at 110° to 120° for one hour before use. Freshly boiled and cooled *water* should be used. The prepared solutions should be stored in chemically resistant, glass-stoppered bottles of alkali-free glass and used within 3 months of preparation. Any solution which has become cloudy or shows any other evidence of deterioration should be discarded.

Standard buffer solutions for various ranges of pH values between 1.2 and 10.0 may be prepared by appropriate combinations of 0.2 N hydrochloric acid or 0.2 N sodium hydroxide and of 0.2 M solutions described below, used in the proportions shown in the accompanying table. The standard pH values given in the table are considered to be reproducible within ± 0.02 Unit at 25°.

1. Hydrochloric acid, 0.2 N : *Hydrochloric acid* diluted with freshly boiled and cooled *water* to contain in 1000 ml 7.292 g of HCl. Standardise as directed under Volumetric Reagents and Solutions.

2. Sodium hydroxide, 0.2 N : Dissolve *sodium hydroxide* in *water* to produce a 40 to 60 per cent w/v solution and allow to stand. Taking precautions to avoid absorption of carbon dioxide, siphon off the clear supernatant liquid and dilute with *carbon dioxide-free water* a suitable volume of the liquid to contain in 1000 ml 8.0 g of NaOH. Standardise as directed under Volumetric Reagents and Solutions.

NOTE — 0.2 N Sodium hydroxide must not be used later than one month after preparation.

3. Potassium hydrogen phthalate, 0.2 M : Dissolve 40.846 g of *potassium hydrogen phthalate* in *water*, and dilute with *water* to 1000 ml.

4. Potassium dihydrogen phosphate, 0.2 M : Dissolve 27.218 g of *potassium dihydrogen phosphate* in *water*, and dilute with *water* to 1000 ml.

5. Boric acid and Potassium chloride, 0.2 M : Dissolve 12.366 g of *boric acid* and 14.911 g of *potassium chloride* in *water*, and dilute with *water* to 1000 ml.

6. Potassium chloride, 0.2 M : Dissolve 14.911 g of *potassium chloride* in *water*, and dilute with *water* to 1000 ml.

COMPOSITION OF STANDARD BUFFER SOLUTIONS

Hydrochloric acid buffer : Place 50 ml of the potassium chloride solution in a 200-ml volumetric flask, add the specified volume of the hydrochloric acid solution, then add *water* to volume.

pH	HCl, ml	pH	HCl, ml
1.2	85.0	1.7	26.0
1.3	67.2	1.8	20.4
1.4	53.2	1.9	16.2
1.5	41.4	2.0	13.0
1.6	32.4	2.1	10.2
		2.2	7.8

Acid phthalate buffer : Place 50 ml of the potassium hydrogen phthalate solution in a 200-ml volumetric flask, add the specified volume of the hydrochloric acid solution, then add *water* to volume.

pH	HCl, ml	pH	HCl, ml
2.2	49.5	3.2	15.7
2.4	42.2	3.4	10.4
2.6	35.4	3.6	6.3
2.8	28.9	3.8	2.9
3.0	22.3	4.0	0.1

Neutralised phthalate buffer : Place 50 ml of the potassium hydrogen phthalate solution in a 200-ml volumetric flask, add the specified volume of the sodium hydroxide solution, then add *water* to volume.

pH	NaOH, ml	pH	NaOH, ml
4.2	3.0	5.2	28.8
4.4	6.6	5.4	34.1
4.6	11.1	5.6	38.8
4.8	16.5	5.8	42.3
5.0	22.6		

Phosphate buffer : Place 50 ml of the potassium dihydrogen phosphate solution in a 200-ml volumetric flask, add the specified volume of the sodium hydroxide solution, then add *water* to volume.

pH	NaOH, ml	pH	NaOH, ml
5.8	3.6	7.0	29.1
6.0	5.6	7.2	34.7
6.2	8.1	7.4	39.1
6.4	11.6	7.6	42.4
6.6	16.4	7.8	44.5
6.8	22.4	8.0	46.1

Alkaline borate buffer : Place 50 ml of the boric acid and potassium chloride solution in a 200-ml volumetric flask, add the specified volume of the sodium hydroxide solution, then add *water* to volume.

pH	NaoH, ml	pH	NaOH, ml
8.0	3.9	9.2	26.4
8.2	6.0	9.4	32.1
8.4	8.6	9.6	36.9
8.6	11.8	9.8	40.6
8.8	15.8	10.0	43.7
9.0	20.8		

NOTE – In this Pharmacopoeia standard buffer solutions are referred to as "buffer solution pH....".

B. Other Buffer Solutions

Acetate buffer pH 2.8 : Dissolve 4 g of *anhydrous sodium acetate* in about 840 ml of *water*, add sufficient *glacial acetic acid* to adjust the pH to 2.8 (about 155 ml) and dilute with *water* to 1000 ml.

Acetate buffer pH 3.5 : Dissolve 25.0 g of *ammonium acetate* in 25 ml of *water* and add 38.0 ml of *7N hydrochloric acid*. Adjust the pH to 3.5 with either *2N hydrochloric acid* or *5N ammonia* and dilute with *water* to 100 ml.

Acetate buffer pH 3.7 : Dissolve 10 g of *anhydrous sodium acetate* in 300 ml of *water*, adjust to pH 3.7 with *glacial acetic acid* and dilute with *water* to 1000 ml. Before use, readjust to pH 3.7, if necessary, with *glacial acetic acid* or *anhydrous sodium acetate*, as required.

Acetate buffer pH 3.9 : Dissolve 15.0 g of *sodium acetate* in 100 ml of *6N acetic acid*.

Acetate buffer pH 4.4 : Dissolve 136 g of *sodium acetate* and 77 g of *ammonium acetate* in *water* and dilute with the same solvent to 1000 ml. Add 250 ml of *glacial acetic acid* and mix.

Acetate buffer pH 4.7 : Dissolve 8.4 g of *sodium acetate* and 3.35 ml of *glacial acetic acid* in sufficient *water* to produce 1000 ml.

Acetate buffer pH 5.0 : Dissolve 13.6 g of *sodium acetate* and 6 ml of *glacial acetic acid* in sufficient *water* to produce 1000 ml.

Acetic-ammonia buffer pH 3.7, ethanolic : To 15 ml of *5N acetic acid* add 60 ml of *alcohol* and 24 ml of *water*. Adjust the pH of the solution to 3.7 with *10N ammonia* and dilute with *water* to 100 ml.

Acetone solution, buffered : Dissolve 8.15 g of *sodium acetate* and 42 g of *sodium chloride* in *water*, add 68 ml of *0.1N hydrochloric acid* and 150 ml of *acetone*, and dilute with *water* to 500 ml.

Ammonia buffer pH 10.0 : Ammonia Buffer Solution. Dissolve 5.4g of *ammonium chloride* in 70 ml of *5N ammonia* and dilute with *water* to 100 ml.

Barbitone buffer pH 8.6 : To 129 ml of *0.1N hydrochloric acid* add sufficient *0.1M barbitone sodium* to produce 1000 ml.

Copper sulphate solution pH 3.2, buffered : Dissolve 7.02 g of *anhydrous disodium hydrogen phosphate* in sufficient *water* to produce 247 ml and add a 2.1 per cent w/v solution of *citric acid* until the pH of the solution is between 3.15 and 3.25 (about 753 ml). Mix 999 ml of the resulting solution with 1 ml of a 0.51 per cent w/v solution of *copper sulphate*.

Copper sulphate solution pH 4.0, buffered : Dissolve 0.25 g of *copper sulphate* and 4.5 g of *ammonium acetate* in sufficient *2N acetic acid* to produce 100 ml.

Glycerin solution pH 7.2, buffered : Determine the pH of a 50 per cent v/v solution of *glycerin* in *carbon dioxide-free water*, Appendix 5.10. If pH of the solution is above 5.5 prepare the buffered glycerin by Method (A), and if pH is below 5.5, prepare by Method (B)

(A) To 500 ml of *glycerin*, add 500 ml of a mixture of 17.5 ml of *M disodium hydrogen phosphate*, 7.5 ml of *M potassium dihydrogen phosphate*, and 475 ml of *carbon dioxide-free water*.

(B) To 500 ml of *glycerin*, add 500 ml of a mixture of 42.0 ml of *M disodium hydrogen phosphate*, 8.0 ml of *M potassium dihydrogen phosphate*, and 450 ml of *carbon dioxide-free water*.

If necessary, adjust the pH to 7.2 with *M disodium hydrogen phosphate* or *M potassium dihydrogen phosphate*, as required. Distribute suitable quantities of the buffered glycerin in screw-capped bottles and sterilise by *Heating in an Autoclave* at 121° for one hour.

Glycine buffer solution : Aminoacetate Buffer Solution. Mix 42 g of *sodium bicarbonate* and 50 g of *potassium bicarbonate* with 180 ml of *water* and add a solution containing 37.5 g of *glycine* and 15 ml of *strong ammonia solution* in 180 ml of *water*. Dilute with *water* to 500 ml and stir until solution is complete.

Imidazole buffer pH 7.4 : Dissolve 3.40 g of *imidazole* and 5.84 g of *sodium chloride* in *water*, add 18.6 ml of *N hydrochloric acid* and dilute with *water* to 1000 ml.

Palladium chloride solution, buffered : To 0.5 g of *palladium chloride* add 5 ml of *hydrochloric acid* and warm on a water-bath. Add 200 ml of hot *water* in small portions with continued heating until solution is complete. Cool and dilute with sufficient *water* to produce 250.0 ml. To 50.0 ml of the resulting solution add 10.0 ml of *M sodium acetate* and 9.6 ml of *N hydrochloric acetate* and sufficient *water* to produce 100.0 ml.

Phosphate buffer pH 4.0, mixed : Dissolve 5.04 g of *disodtum hydrogen phosphate* and 3.01 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml and adjust the pH to 4.0 with *glacial acetic acid*.

Phosphate buffer pH 6.8, mixed : Dissolve 28.80 g of *disodium hydrogen phosphate* and 11.45 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml.

Phosphate buffer pH 7.0, mixed : Dissolve 0.50 g of *anhydrous disodium hydrogen phosphate* and 0.301 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml.

Phosphate-buffered saline pH 7.4 : Dissolve 1.38 g of *disodium hydrogen phosphate*, 0.19 g of *potassium dihydrogen phosphate* and 8.0 g of *sodium chloride* in sufficient *water* to produce 1000 ml. Immediately before use adjust the pH, if necessary.

7.2 COLORIMETRIC SOLUTIONS

Colorimetric solutions are used in the preparation of standards for the measurement of colour of certain pharmacopoeial substances and also for the limit test for readily carbonisable substances, specified in some monographs. These solutions should be prepared and standardised in the manner described, and stored in suitable resistant, tightly-closed containers.

Cobalt Chloride C. S. – Synonym – **Cobaltous Chloride Colorimetric Solution**

Dissolve about 65 g of *cobalt chloride*, in enough of a mixture of 25 ml of *hydrochloric acid* and 975 ml of *water* to make 1000 ml. Pipette 5 ml of this solution in a 250-ml iodine flask, add 5 ml of *hydrogen peroxide solution* and 15 ml of *sodium hydroxide solution*, boil for ten minutes, cool and add 2 g of *potassium iodide* and 60 ml of *dilute sulphuric acid*. When the precipitate has dissolved, titrate the liberated iodine with *0.1N sodium thiosulphate* using *starch solution* as indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.02379 g of $CoCl_2, 6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of *hydrochloric acid* and *water* so that each ml contain 0.0595 g of $CoCl_2, 6H_2O$.

Copper Sulphate C. S. – Synonym – **Cupric Sulphate Colorimetric Solution**

Dissolve about 65 g of *copper sulphate* in enough of a mixture of 25 ml of *hydrochloric acid* and 975 ml of *water* to make 1000 ml. Pipette 10 ml of this solution into a 250-ml of iodine flask, add 40 ml of *water*, 4 ml of *acetic acid*, 3 g of *potassium iodide*, and 5 ml of *hydrochloric acid* and titrate the liberated iodine with *0.1N sodium thiosulphate*, adding 3 ml of *starch solution* as indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.02497 g of $CuSO_4, 5H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of *hydrochloric acid* and *water* so that each ml contains 0.0624 g of $CuSO_4, 5H_2O$.

Ferric Chloride C. S. – Synonym – **Ferric Chloride Colorimetric Solution**

Dissolve about 55 g of *ferric chloride hexahydrate* in enough of a mixture of 25 ml of *hydrochloric acid* and 975 ml of *water* to produce 1000 ml. Pipette 10 ml of this solution into a 250-ml iodine flask, add 15 ml of *water*, 3 g of *potassium iodide*, and 5 ml of *hydrochloric acid*, and allow the mixture to stand for fifteen minutes. Dilute with 100 ml of *water*, and titrate the liberated iodine with *0.1N sodium thiosulphate*, adding 3 ml of *starch solution* as indicator. Perform a blank determination with the same quantities of the same reagents, and make any necessary correction. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.02703 g of $FeCl_3, 6H_2O$. Adjust the final volume

of the solution by the addition of enough of the mixture of *hydrochloric acid* and *water* so that each ml contains 0.045 g of $FeCl_3, 6H_2O$.

7.3 INDICATORS AND INDICATOR TEST PAPERS

A. Indicators

In the tests and assays of the Pharmacopoeia, indicators are required to indicate the completion of a chemical reaction in volumetric analysis or to indicate the pH of solutions. Given below are materials which are to be used as indicators and the manner in which solutions of indicators are to be prepared. Also listed in ascending order of the lower limit of their range, are pH indicators with the corresponding colour changes.

Alizarin yellow GG : CI 14025; Mordant Yellow; $C_{13}H_8N_3NaO_5 = 309.2$.

Gives a pale-yellow colour in weakly alkaline solutions, and a brownish-yellow colour in more strongly alkaline solutions (pH range, 10.0 to 12.0).

Alizarin yellow GG solution

A 0.1 per cent w/v solution of *alizarin yellow GG*.

Azo violet : Magneson; 4-(4-Nitrophenylazo)resorcinol; $C_{12}H_9N_3O_4 = 259.2$

Red powder. It melts at about 193° with decomposition, Appendix 5.11. When used for titration in non-aqueous media, it changes from orange (acidic) through pink to blue (basic).

Azo violet solution (Magneson reagent)

A 0.1 per cent w/v solution of *azo violet* in a 1 per cent w/v solution of *sodium hydroxide*.

Bromocresol green : 4, 4'-(3*H*-2, 1-Benzoxathiol-3-ylidene)bis (2,6-dibromo-*m*-cresol) *SS*-dioxide $C_{21}H_{14}Br_4O_5S = 698$.

Gives a yellow colour in moderately acid solutions, and a blue colour in weakly acid and alkaline solutions (pH range, 3.6 to 5.2).

Bromocresol green solution

Warm 0.1 g of *bromocresol green* with 2.9 ml of *0.05N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add a sufficient quantity of *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.2 ml of the solution and 100 ml of *carbon dioxide-free water* is blue. Not more than 0.20 ml of *0.02N hydrochloric acid* is required to change the colour to yellow.

Bromocresol purple : 4,4'-(3*H*-2,1-Benzoxathiol-3-ylidene)bis (2,6-dibromo-*o*-cresol) *SS*-dioxide; Dibromo-*o*-cresolsulphonphthalein; $C_{21}H_{16}Br_2O_5S = 540.2$.

Gives a yellow colour in weakly acid solutions and a bluish-violet colour in alkaline, neutral and extremely weakly acid solutions (pH range, 5.2 to 6.8).

Bromocresol purple solution

Warm 0.1 g of *bromocresol purple* with 5 ml of *alcohol (90 per cent)* until dissolved, add 100 ml of *alcohol (20 per cent)*, 3.7 ml of *0.05N sodium hydroxide*, and sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.2 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.05 ml *0.02N sodium hydroxide* has been added is bluish-violet. Not more than 0.20 ml of *0.02N hydrochloric acid* is required to change the colour to yellow.

Bromophenol blue : 4, 4'-(3*H*-2, 1-Benzoxathiol-3-ylidene)bis-(2, 6-dibromophenol) *SS* dioxide; $C_{19}H_{10}Br_4O_5S = 670$

Gives a yellow colour in moderately acid solutions, and a bluish-violet colour in weakly acid and alkaline solutions (pH range, 2.8 to 4.6).

Bromophenol blue solution

Warm 0.1 g of of *bromophenol blue* with 3.0 ml of *0.05N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected add sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.05 ml of the solution and 20 ml of *carbon dioxide-free water* to which 0.05 ml of *0.1N hydrochloric acid* has been added is yellow. Not more than 0.10 ml of *0.1N sodium hydroxide* is required to change the colour to bluish-violet.

Bromothymol blue : 6,6'-(3*H*-2,1-Benzoxathiol-3-ylidene)bis(2-bromothymol) *SS*-dioxide; $C_{27}H_{28}Br_2O_5S = 624$

Gives a yellow colour in weakly acid solutions and a blue colour in weakly alkaline solutions. Neutrality is indicated by a green colour (pH range, 6.0 to 7.6).

Bromothymol blue solution

Warm 0.1 g of *bromothymol blue* with 3.2 ml of *0.05N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.3 ml of the solution and 100 ml of *carbon dioxide-free water* is yellow. Not more than 0.10 ml of *0.02N sodium hydroxide* is required to change the colour to blue.

Calcon : CI 15705; Sodium 2-hydroxy-1-(2-hydroxy-1-

naphthylazo) naphthalene-4-sulphonate; Solochrome Dark Blue; Mordant Black 17; $C_{20}H_{13}N_2NaO_5S = 416.4$

A brownish-black powder with a violet sheen. Gives a purple-red colour with calcium ions in alkaline solution. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediaminetetraacetate, the solution is blue.

Calcon mixture

Mix one part of *calcon* with 99 parts of *sodium chloride*.

SENSITIVITY – Dissolve 50 mg of *calcon mixture* in a mixture of 100 ml of *water* and 4 ml of *sodium hydroxide solution*. The solution is blue but becomes violet or addition of 1 ml of a 1 per cent w/v solution of *magnesium sulphate* and 0.1 ml of a 0.15 per cent w/v solution of *calcium chloride* and turns pure blue on addition of 0.15 ml of *0.01M disodium ethylenediaminetetraacetate*.

Catechol violet : 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)-dipyrocatechol *SS*-dioxide

Gives a blue colour with bismuth ions in moderately acid solution. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediaminetetraacetate, the solution is yellow.

Catechol violet solution

Dissolve 0.1 g of *catechol violet* in 100 ml of *water*.

Congo red : CI 22120; Sodium 4,4′-diphenyl-bis(2-azo-1-naphthylamine-4-sulphonate); $C_{32}H_{22}N_6Na_2O_6S_2 = 696.66$

Gives a blue colour in moderately acid solutions, and a red colour in weakly acid and alkaline solutions (pH range, 3.0 to 5.0).

Congo red fibrin : Soak washed and shredded *fibrin* overnight in a 2.0 per cent w/v solution of *congo red* in *alcohol (90 per cent)*; strain, wash the product with water, and store under *solvent ether*.

Congo red solution

Dissolve 0.25 g of *congo red* in 50 ml of *alcohol (90 per cent)* and add sufficient *water* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.2 ml of the solution, 100 ml of *carbon dioxide-free water* and 0.3 ml of *0.1N hydrochloric acid* is blue. Not more than 0.3 ml of *0.1N sodium hydroxide* is required to change the colour to pink.

Cresol red : 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)di-*o*-cresol *SS*-dioxide; $C_{21}H_{18}O_5S = 382.4$

Gives a red colour in very strongly acid solutions, a yellow colour is less strongly acid and neutral solutions, and a red colour in moderately alkaline solutions (pH range, 0.2 to 1.8 and 7.2 to 8.8).

Cresol red solution

Warm 50 mg of *cresol red* with 2.65 ml of *0.05N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.15 ml of *0.15N sodium hydroxide* has been added is purplish-red. Not more than 0.15 ml of *0.02N hydrochloric acid* is required to change the colour to yellow.

Crystal-violet : CI 42555; CI Basic Violet 3; Hexamethyl-*p*-rosaniline Chloride; $C_{25}H_{30}ClN_3 = 408.0$

When used for titration in non-aqueous media, changes from violet (basic) through blue-green (neutral) to yellowish green (acidic).

Crystal-violet solution

A 0.5 per cent w/v solution of *crystal-violet* in *glacial acetic acid*.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml of the solution and 50 ml of *glacial acetic acid* is bluish-purple. Add 0.10 ml of a solution of *0.1N perchloric acid*; the solution turns blue-green.

Dimethyl yellow : CI 11020; 4-Dimethylaminoazobenzene; $C_{14}H_{15}N_3 = 225.3$

Gives a red colour in moderately acid alcoholic solutions, and a yellow colour in weakly acid and alkaline solution (pH range, 2.8 to 4.6).

Complies with the following test:

HOMOGENEITY – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and *methylene chloride* as the mobile phase. Apply to the chromatoplate 10 µl of a 0.01 per cent w/v solution in *methylene chloride*; the chromatogram shows only one spot.

Dimethyl yellow solution

A 0.2 per cent w/v solution of *dimethyl yellow* in *alcohol (90 per cent)*.

Complies with the following test:

SENSITIVITY – A solution containing 2 g of *ammonium chloride* in 25 ml of *carbon dioxide-free water*, to which is added 0.1 ml of the dimethyl yellow solution, is yellow. Not more than 0.10 ml of *0.1N hydrochloric acid* is required to change the colour to red.

Dimethyl yellow-oracet blue B solution : Dimethyl yellow-solvent blue 19 solution

Dissolve 15 mg of *dimethyl yellow* and 15 mg of *oracet blue B* in *chloroform* and dilute with *chloroform* to 500 ml.

Eriochrome black T : CI 14645; Mordant Black 11; Sodium 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulphonate; $C_{20}H_{12}N_3NaO_7S = 461.38$

Brownish black powder having a faint, metallic sheen. Soluble in *alcohol*, in *methyl alcohol* and in hot *water*.

Complies with the following test:

SENSITIVITY – To 10 ml of a 1 in 200,000 solution in a mixture of equal parts of *methyl alcohol* and *water* add a 1 per cent w/v solution of *sodium hydroxide* until the pH is 10; the solution is pure blue in colour and free from cloudiness. Add 0.01 ml of *0.05M magnesium sulphate*; the colour of the solution changes to red-violet, and on adding more *0.05M magnesium sulphate*, the solution becomes wine-red in colour.

Eriochrome black T solution

Dissolve 0.2 g of *eriochrome black T* and 2 g of *hydroxylamine hydrochloride* in sufficient *methyl alcohol* to produce 50 ml.

Prepare immediately before use.

Ferric ammonium sulphate solution

A 10 per cent w/v solution of *ferric ammonium sulphate* in *water*.

Gives a deep red colour with ammonium thiocyanate in acid solutions.

Ferroin sulphate solution : Ortho-phenanthroline ferrous complex; Tris(1,10-phenanthroline)-ferrous sulphate complex

Dissolve 0.7 g of *ferrous sulphate* in about 70 ml of *water* and add 1.5 g of *1,10-phenanthroline* and sufficient *water* to produce 100 ml.

Complies with the following test:

SENSITIVITY – Add 0.1 ml of the solution and 0.15 ml of *osmic acid solution* to 50 ml of *2N sulphuric acid*. Add 0.1 ml of *0.1N ceric ammonium sulphate*; the colour changes from red to light blue.

Hydroxy naphthol blue indicator : Disodium salt of 1-(2-naphtholazo-3, 6-disulphonic acid)-2-naphthol-4-sulphonic acid deposited on crystals of sodium chloride; $C_{20}H_{14}N_2O_{11}S_3 = 554.52$

Small blue crystals freely soluble in *water*. In the pH range between 12 to 13, its solution is reddish-pink in the presence of calcium ions and deep blue in the presence of excess disodium ethylenediaminetetraacetate.

Complies with the following test:

SENSITIVITY – Dissolve 0.3 g in 100 ml of *water*, add 10 ml of *N sodium hydroxide* and 1.0 ml of a 0.5 per cent w/v solution of *calcium chloride*, and dilute to 165 ml with water; the solution is reddish-pink in colour. Add 1.0 ml of *0.05M disodium ethylenediaminetetraacetate*; the solution becomes deep blue in colour.

Light green SF yellowish : CI 42095; Acid Green 5; Acid Green G; Food Green 2, $C_{37}H_{34}N_2Na_2O_9S_3 = 792.9$.

A reddish-brown powder. An oxidation-reduction indicator which changes from bluish-green to colourless on reduction.

Light green SF yellowish solution

A 0.1 per cent w/v solution of *light green SF yellowish solution* in *water*.

Litmus

Fragments of blue pigment prepared from various species of *Rocella, Lecanora* or other lichens. It has a characteristic odour.

Partly soluble in *water* and in *alcohol*. Gives a red colour with acids and a blue colour with alkalis (pH range, 5.0 to 8.0).

NOTE – Litmus is unsuitable for determining the pH of alkaloids, carbonates and bicarbonates.

Litmus solution

Boil 25 g of coarsely powdered *litmus* with 100 ml of *alcohol (90 per cent)* under a reflux condenser for one hour, and pour away the clear liquid; repeat this operation using two successive quantities, each of 75 ml, of *alcohol (90 per cent)*. Digest the extracted litmus with 250 ml of *water*.

Metacresol purple : 4,4'-(3*H*-2,1-Benzoxathiol-3-ylidene)di-*m*-cresol *SS*-dioxide; $C_{21}H_{16}O_5S = 380.4$

Gives a red colour in strongly acid solutions, a yellow colour in less strongly acid and neutral solutions, and a violet colour in moderately alkaline solutions (pH range, 0.5 to 2.5 and 7.5 to 9.2).

Metacresol purple solution

A 0.1 per cent w/v solution of *metacresol purple* in *0.001N sodium hydroxide*.

Metanil yellow : CI 13065; Sodium 4'-anilinoazobenzene-3-sulphonate; $C_{18}H_{14}N_3NaO_3S = 375.4$

When used for titration in non-aqueous media, it changes from yellow (basic) into magenta (acidic).

Metanil yellow solution

A 0.1 per cent w/v solution of *metanil yellow* in *methyl alcohol*.

Complies with the following test:

SENSITIVITY – A solution containing 0.1 ml in 50 ml of *glacial acetic acid* is pinkish-red. Add 0.05 ml of *0.1N perchloric acid*; the colour changes to violet.

Methyl orange : CI 13025; Sodium 4'-dimethylaminoazobenzene-4-sulphonate; $C_{14}H_{14}N_3NaO_3S = 327.3$

Gives a red colour in moderately acid solutions, and a yellow colour in weakly acid and alkaline solutions (pH range, 2.9 to 4.0).

Methyl orange solution

A 0.1 per cent w/v solution of *methyl orange* in *alcohol (20 per cent)*.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml of the solution and 100 ml of *carbon dioxide-free water* is yellow; not more than 0.1 ml of *0.1N hydrochloric acid* is required to change the colour to red.

Methyl orange-bromocresol green solution

Dissolve 20 mg of *methyl orange* and 0.1 g of *bromocresol green* in 1 ml of *0.2N sodium hydroxide* and dilute with *water* to 100 ml.

Gives an orange colour in moderately acid solutions, and an olive-green colour in weakly acid and alkaline solutions (pH range, 3.0 to 4.4).

Methyl orange-xylene cyanol FF solution

Dissolve 0.1 g of *methyl orange* and 0.26 g of *xylene cyanol FF* in 50 ml of *alcohol* and add sufficient *water* to produce 100 ml.

Methyl red : CI 13020; 4′-Dimethylaminoazobenzene-2-carboxylic acid; $C_{15}H_{15}N_3O_2$ = 269.3

Gives a red colour in weakly acid solutions and a yellow colour in very weakly acid and alkaline solutions (pH range, 4.2 to 6.3).

Methyl red solution

Warm 0.1 g of *methyl red* with 1.85 ml of *0.2N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add sufficient *alcohol (50 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml, 100 ml of *carbon dioxide-free water* and 0.05 ml of *0.02N hydrochloric acid* is red; not more than 0.1 ml of *0.02N sodium hydroxide* is required to change the colour to yellow.

Methyl red solution, alcoholic

Dissolve 1 g of *methyl red* in 100 ml of *ethyl alcohol* and filter, if necessary.

Methyl red-methylene blue solution

Mix 20 ml of a 0.05 per cent w/v solution of *methyl red* in *alcohol (80 per cent)* with 0.4 ml of a 2.0 per cent w/v solution of *methylene blue* in *water*.

Methyl thymol blue : [3 *H* - 2, 1 - Benzoxathiol - 3 - ylidenebis (6 - hydroxy - 5 - isopropyl - 2 - methyl - *m* - phenylene) methylenenitrilo]tetra - acetic acid *SS*- dioxide; $C_{37}H_{40}N_2Na_4O_{13}S$ = 845.0

Gives a blue colour with calcium in alkaline solutions. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediaminetetraacetate, the solution is grey.

Methyl thymol blue mixture

A mixture of 1 part of *methyl thymol blue* and 100 parts of *potassium nitrate*.

Mordant black 11 : See *Eriochrome black T*.

Mordant black 11 mixture : Mordant black mixture A mixture of 0.2 part of *mordant black 11* with 100 parts of *sodium chloride*. Mordant Black 11 Mixture should be recently prepared.

Mordant Blue 3 : CI 43820; Solochrome cyanine R A dark red, or reddish-brown powder

Gives an intense purple colour with aluminium in weakly acid solutions. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediaminetetraacetate, the solution is pale pink.

Mordant red 7 : CI 18760; Sodium2-hydroxy-1-(5-hydroxy-3-methyl-1-phenyl-4-pyrazolylazo)-2-naphthalein-4-sulphonate

Gives a bluish-violet colour with nickel. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediamine tetraacetate, the solution is green.

Mordant red 7 solution

Dissolve 0.1 g of *mordant red 7* in 100 ml of *water*.

1-Naphtholbenzein : α-Naphtholbenzein; Phenylbis (4-hydroxynaphthyl)-methanol; $C_{27}H_{20}O_3$ = 392.5

When used for titration in non-aqueous media, it changes from blue or green-blue (basic) through orange (neutral) to dark green (acidic).

1-Naphtholbenzein solution : α-Naphtholbenzein solution

A 0.2 per cent w/v solution of *1-naphtholbenzein* in *glacial acetic acid*.

Complies with the following test:

SENSITIVITY – Add 0.25 ml to 50 ml of *glacial acetic acid*. Not more than 0.05 ml of *0.1N perchloric acid* is required to change the colour of the solution from brownish-yellow to green.

1-Naphtholphthalein : α-Naphtholphthalein; 3,3-bis (4-hydroxyl-1-naphthyl)-phthalide; $C_{28}H_{18}O$ = 418.4

Gives a colourless solution or a pale red colour in acid and neutral solutions and a blue colour in moderately alkaline solutions (pH range, 7.3 to 8.7).

1-Naphtholphthalein solution : α-Naphtholphthalein solution

A 0.5 per cent w/v solution of *1-naphtholphthalein* in *alcohol*.

Neutral red : CI 50040; CI Basic red; 3-Amino-7-dimethylamino-2-methylphenazine monohydrochloride; $C_{15}H_{16}N_4$,HCl = 288.78

Reddish to olive-green, coarse powder. Sparingly soluble in *water* and in *alcohol*.

Gives a red colour with acids and an orange colour with alkalis (pH range, 6.8 to 8.0).

Neutral red solution : A 0.1 per cent w/v solution of *neutral red* in *alcohol (50 per cent)*.

Oracet blue B : Solvent blue 19

A mixture of 1-methyl-amino-4-anilinoanthraquinone ($C_{21}H_{16}N_2O_2$) and 1-amino-4-anilinoanthraquinine ($C_{20}H_{14}N_2O_2$).

When used for titration in non-aqueous media, it changes from blue (basic) through purple (neutral) to pink (acidic).

Oracet blue B solution : Solvent Blue 19 solution

A 0.5 per cent w/v solution of *oracet blue B in glacial acetic acid*.

Orthophenanthroline solution : Dissolve 1.5 g of *1,10-phenanthroline* in sufficient *water* to produce 100 ml.

Phenol red : 4,4′-(3*H*-2,1-Benzoxathiol-3-ylidene)diphenol *SS*-dioxide; $C_{19}H_{14}O_5S = 354.4$

Gives a yellow colour in neutral and very faintly acid solutions and a red colour in weakly alkaline solutions (pH range, 6.8 to 8.4)

Phenol red solution

Warm 0.1 g of *phenol red* with 1.42 ml of *0.2N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml and 100 ml of *carbon dioxide-free water* is yellow. Not more than 0.1 ml of *0.02N sodium hydroxide* is required to change the colour of the solution to reddish-violet.

Phenolphthalein : 3, 3 - Bis (4 - hydroxyphenyl) phthalide; $C_{20}H_{14}O_4 = 318.3$

Gives a colourless solution in acid and weakly alkaline solutions and gives a red colour in more strongly alkaline solutions (pH range, 8.3 to 10.0).

Phenolphthalein solution

A 1.0 per cent w/v solution of *phenolphthalein* in *alcohol (50 per cent)*.

Phenolphthalein solution, dilute

A 0.1 per cent w/v solution of *phenolphthalein* in *alcohol (80 per cent)*.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml and 100 ml of *carbon dioxide-free water* is colourless. Not more than 0.2 ml of *0.02N sodium hydroxide* is required to change the colour to pink.

Phenolphthalein-thymol blue solution

Dissolve 0.1 g of *thymol blue* in a mixture of 2.2 ml of *0.1N sodium hydroxide* and 50 ml of *alcohol* and dilute with *water* to 100 ml. Mix 3 volumes of this solution with 2 volumes of a 0.1 per cent w/v solution of *phenolphthalein* in *alcohol (80 per cent)*.

Potassium chromate solution

A 5.0 per cent w/v solution of *potassium chromate*.

Gives a red precipitate with silver nitrate in neutral solutions.

Pyridylazonaphthol : PAN; 1-(2-pyridylazo)-2-naphthol; $C_{15}H_{11}N_3O = 249.3$

A brick-red powder; it melts between 137° and 140°, Appendix 5.11. Gives a red colour with copper ions; when metal ions are absent, for example, in the presence of an excess of *disodium ethylenediaminetetraacetate*, the solution is yellow.

Complies with the following test:

SENSITIVITY – To 50 ml of *water*, add 10 ml of *acetate buffer pH 4.4*, 0.1 ml of *0.02M disodium ethylenediamine tetraacetate* and 0.25 ml of a 0.1 per cent w/v solution of the pyridylazonaphthol in *alcohol*; the solution is yellow. Add 0.15 ml of a 0.5 per cent w/v solution of *copper sulphate*; the colour of the solution changes to red.

Pyridylazonaphthol solution

A 0.1 per cent w/v solution of *pyridylazonaphthol* in *alcohol*.

Quinaldine red : 2-(4-Dimethylaminostyryl)quinoline ethiodide; $C_{21}H_{23}IN_2 = 430.33$

When used for the titration of bases with perchloric acid in glacial acetic acid, changes from magenta to almost colourless.

Quinaldine red solution

A 0.1 per cent w/v solution of *quinaldine red* in *methyl alcohol*.

Starch solution : Starch mucilage

Triturate 0.5 g of *starch* or *soluble starch* with 5 ml of *water*, and add this, with constant stirring, to sufficient *water* to produce about 100 ml. Boil for a few minutes, cool and filter.

Gives a blue colour with free iodine in the presence of a soluble iodide.

NOTE – Starch solution must be freshly prepared.

Thymol blue : 6,6′-(3*H*-2,1-Benzoxathiol-3-ylidene)dithymol *SS*-dioxide; $C_{27}H_{30}O_5S = 466.6$

Gives a red colour in strongly acid solutions, a yellow colour in weakly acid and weakly alkaline solutions, and a blue colour in more strongly alkaline solutions (pH range, 1.2 to 2.8 and 8.0 to 9.6).

Thymol blue solution

Warm 0.1 g of *thymol blue* with 4.3 ml of *0.05N sodium hydroxide* and 5 ml of *alcohol (90 per cent)*; after solution is effected, add sufficient *alcohol (20 per cent)* to produce 250 ml.

Complies with the following test:

SENSITIVITY – A mixture of 0.1 ml and 100 ml of *carbon dioxide-free water* to which 0.02 ml of *0.02N sodium hydroxide* has been added is blue. Not more than 0.1 ml of *0.2N hydrochloric acid* is required to change the colour to yellow.

Thymol blue solution, alcoholic

Dissolve 100 mg of *thymol blue* in 100 ml of *alcohol* and filter, if necessary.

Thymolphthalein : 3, 3 - Bis (4 - hydroxy - 5 - isopropyl - 2-methylphenyl)phthalide; $C_{28}H_{30}O_4$ = 430.5

Forms a colourless solution in acid and weakly alkaline solutions, and gives a blue colour in more strongly alkaline solutions (pH range, 9.3 to 10.5).

Thymolphthalein solution

A 0.1 per cent w/v solution of *thymolphthalein* in *alcohol*.

Complies with the following test:

SENSITIVITY – A mixture of 0.05 ml and 100 ml of *carbon dioxide-free water* is colourless. Not more than 0.05 ml of *0.1N sodium hydroxide* is required to change the colour to blue.

Titan yellow : CI 19540; Thiazole yellow; Sodium 2,2'-[(diazoamino) di-*p*-phenylene] bis-(6-methylbenzothiazole-7-sulphonate); $C_{28}H_{19}N_5Na_2O_6S_4$ = 696

Gives a yellow colour in acid and moderately alkaline solutions and a red colour in strongly alkaline solutions (pH range, 12.0 to 13.0).

Titan yellow solution

A 0.05 per cent w/v solution of *titan yellow*.

Complies with the following test:

SENSITIVITY – Add 0.1 ml to a mixture of 10 ml of *water*, 0.2 ml of a 0.0101 per cent w/v solution of *magnesium sulphate* and 1.0 ml of *N sodium hydroxide*; a pink colour is produced.

Xylene cyanol FF : CI 42135; Sodium salt of 4',4''-bis (ethylamino)-3', 3''-dimethyltriphenylmethano-2, 4-disulphonic anhydride

A blue alcohol-soluble dye used as a screening agent in *methyl orange-xylene cyanol FF solution*.

Xylenol orange : [3*H*-2,1-Benzoxathiol-3-ylidene bis-(6-hydroxy - 5 - methyl - *m*- phenylene) methylenenitrilo]tetraacetic acid *SS*-dioxide ($C_{31}H_{32}N_2O_{13}S$) or its tetra-sodium salt.

Gives a violet colour with mercury, lead, zinc, and certain other metal ions in acid solution. When metal ions are absent, for example, in the presence of an excess of disodium ethylenediamine tetraacetate, this solution is yellow

Xylenol orange mixture

Triturate 1 part of *xylenol orange* with 99 parts of *potassium nitrate*.

Complies with the following test:

SENSITIVITY – Add 50 mg to a mixture of 50 ml of *water*, 1 ml of *2N acetic acid* and 0.05 ml of *0.1M lead nitrate*. Add sufficient *hexamine* to change the colour to violet, and add 0.1 ml of *0.1M disodium ethylenediaminetetraacetate*; the colour changes to yellow.

Xylenol orange solution

Shake 0.1 g of *xylenol orange* with 100 ml of *water* and filter, if necessary.

B. Indicators and Test Papers

Indicator and test papers are strips of filter paper of suitable dimension and grade impregnated with an indicator or reagent that is sufficiently stable to provide a convenient form of the impregnated substance. Commercial samples of indicator and test papers are available and may be used. Those required in the assays and tests of the Pharmacopoeia may be prepared as described in the following paragraphs:

Treat strong, white filter paper with *hydrochloric acid*, and wash with *water* until the last washing does not show an acid reaction to *methyl red*. Then treat with *dilute ammonia solution* and wash again with *water* until the last washing is not alkaline to *phenolphthalein*. Dry the paper thoroughly and saturate it with the proper strength of the indicator solution and dry carefully in still air, by suspending it from glass rods in a space free from acid and other fumes. Cut the paper into strips of suitable size, and store in well-closed containers, protected from light and moisture.

Congo red paper : Prepare from *congo red solution* and dry the impregnated paper.

Lead acetate paper : Prepare from *lead acetate solution* and dry the impregnated paper at 100°, avoiding contact with metal.

Litmus paper, blue : Boil 10 parts of coarsely powdered *litmus* under reflux for one hour with 100 parts of *alcohol*, decant the alcohol and discard. Add to the residue a mixture of 45 parts of *alcohol* and 55 parts of *water*. After two days decant the clear liquid. Impregnate strips of filter paper with the extract and allow to dry. The paper complies with the following test:

SENSITIVITY – Immerse a strip measuring 10 mm × 60 mm in 100 ml of a mixture of 10 ml of *0.02N hydrochloric acid* and 90 ml of *water*. On shaking, the paper turns red within forty-five seconds.

pH Ranges and Colour Changes of Indicators

Indicator	pH Range	Colour Change
Cresol Red	0.2 to 1.8	Red to Yellow
	and	
	7.2 to 8.8	Yellow to Red
Metacresol Purple	0.5 to 2.5	Red to Yellow
	and	
	7.5 to 9.2	Yellow to Violet
Thymol Blue	1.2 to 2.8	Red to Yellow
	and	
	8.0 to 9.6	Yellow to Violet-blue
Metanil Yellow	1.2 to 3.2	Red to Yellow
Quinaldine Red	1.4 to 3.2	Colourless to Red
Naphthol Yellow	2.0 to 3.2	Colourless to Yellow
Dimethyl Yellow	2.8 to 4.6	Red to Yellow
Bromophenol Blue	2.8 to 4.6	Yellow to Blue-violet
Methyl Orange	2.9 to 4.0	Red to Yellow
Methyl Orange-xylene cyanol FF	2.9 to 4.6	Violet to Green
Congo Red	3.0 to 5.0	Blue to Red
Bromocresol Green	3.6 to 5.2	Yellow to Blue
Methyl Red	4.2 to 6.3	Red to Yellow
Litmus	5.0 to 8.0	Red to Blue
Bromocresol Purple	5.2 to 6.8	Yellow to Blue-violet
Bromothymol Blue	6.0 to 7.6	Yellow to Blue
Neutral Red	6.8 to 8.0	Red to Orange
Phenol Red	6.8 to 8.4	Yellow to Red
1-Naphtholphthalein	7.3 to 8.7	Pale red to Blue
Phenolphthalein	8.3 to 10.0	Colourless to Red
Thymolphthalein	9.3 to 10.5	Colourless to Blue
Alizarin Yellow GG	10.0 to 12.0	Colourless to Yellow
Titan Yellow	12.0 to 13.0	Yellow to Red

Litmus paper, red : To the extract obtained in the preparation of *blue litmus paper* add *2N hydrochloric acid* dropwise until the blue colour becomes red. Impregnate strips of filter paper with the solution and allow to dry.

The paper complies with the following test:

SENSITIVITY – Immerse a strip measuring 10 mm × 60 mm in 100 ml of *0.002N sodium hydroxide*. On shaking, the paper turns blue within forty-five seconds.

pH-Indicator paper, narrow range : Use a suitable commercial grade of the specific range of pH required.

Phenolphthalein paper : Prepare from *dilute phenolphthlein solution* and dry the impregnated paper.

Starch-iodate paper : Prepare from a mixture of equal volumes of *starch solution* and a 5 per cent w/v solution of *potassium iodate* in *water* and dry the impregnated paper.

Starch-iodide paper : Prepare from a mixture of equal volumes of *starch solution* and 5 per cent w/v solution of *potassium iodide* in *water* and dry the impregnated paper.

Titan yellow paper : Prepare from *titan yellow solution* and dry the impregnated paper.

Turmeric paper : Use a solution prepared as follows: Macerate 20 g of powdered *turmeric* with four quantities, each of 100 ml, of *water*, decant the clear supernatant liquid each time and discard it. Dry the residue at a temperature not exceeding 100°. Macerate with 100 ml of *alcohol* for several days and filter. The impregnated and dried paper complies with the following test:

Dip a strip, about 1.5 cm in length, in a solution of 1.0 mg of *boric acid* in 5 ml of *water*, previously mixed with 1 ml of *hydrochloric acid*. After one minute remove the paper from the liquid, and allow it to dry; the yellow colour changes to brown. Then moisten the paper with *dilute ammonia solution*; the colour of the paper changes to greenish-black.

7.4 GENERAL REAGENTS

Acacia

Of the Indian Pharmacopoeia

Acetaldehyde : $CH_3CHO = 44.05$

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

SOLUBILITY – Miscible with *water*, with *alcohol*, with *solvent ether*, and with *benzene*.

DISTILLATION RANGE – Not less than 95 per cent distils between 20° and 22°, Appendix 5.3.

WT. PER ML – At 20°, 0.783 to 0.784 g, Appendix 5.19.

ACIDITY – To 10.0 g add sufficient *carbon dioxide-free water* to produce 50 ml and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator; not more than 5.0 ml of *0.1N sodium hydroxide* is required.

Acetic Acid

Contains approximately 33 per cent w/v of $C_2H_4O_2$. Dilute 315 ml of *glacial acetic acid* to 1000 ml with *water*.

Acetic Acid, *x*N

Solutions of any normality *x*N may be prepared by diluting 60*x* ml of *glacial acetic acid* to 1000 ml with *water*.

Acetic Acid, Dilute

Contains approximately 6 per cent w/w of $C_2H_4O_2$. Dilute 57 ml of *glacial acetic acid* to 1000 ml with *water*.

Acetic Acid Glacial : $CH_3.COOH = 60.05$

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$. About 17.5 N in strength.

DESCRIPTION – At a temperature above its freezing point a clear, colourless liquid; odour, pungent and characteristic; crystallises when cooled to about 10° and does not completely remelt until warmed to about 15°.

SOLUBILITY – Miscible with *water*, with *alcohol*, with *glycerin* and with most fixed and volatile oils.

BOILING RANGE – Between 117° and 119°, Appendix 5.3.

CONGEALING TEMPERATURE – Not lower than 14.8°, Appendix 5.5.

WT. PER ML – At 25°, about 1.047 g, Appendix 5.19.

ARSENIC – Not more than 2 parts per million, Appendix 3.2.1.

HEAVY METALS – Evaporate 5 ml to dryness in a porcelain dish on a water-bath, warm the residue with 2 ml of *0.1N hydrochloric acid* and add *water* to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 3.2.4.

CHLORIDE – 5 ml complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 5 ml complies with the *limit test for sulphates*, Appendix 3.2.8.

CERTAIN ALDEHYDIC SUBSTANCES – To 5 ml add 10 ml of *mercuric chloride solution*, and make alkaline with *sodium hydroxide solution*, allow to stand for five minutes and acidify with *dilute sulphuric acid*; the solution does not show more than a faint turbidity.

FORMIC ACID AND OXIDISABLE IMPURITIES – Dilute 5 ml with 10 ml of *water*; to 5 ml of this solution add 2.0 ml of *0.1N potassium dichromate* and 6 ml of *sulphuric acid*, and

allow to stand for one minute, add 25 ml of *water*, cool to 15°, and add 1 ml of freshly prepared *potassium iodide solution* and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Not less than 1 ml of *0.1N sodium thiosulphate* is required.

ODOROUS IMPURITIES – Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

READILY OXIDISABLE IMPURITIES – To 5 ml of the solution prepared for the test for **FORMIC ACID AND OXIDISABLE IMPURITIES**, add 20 ml of *water* and 0.5 ml of *0.1N potassium permanganate*; the pink colour does not entirely disappear within half a minute.

NON-VOLATILE MATTER – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105°.

ASSAY – Weigh accurately about 1 g into a stoppered flask containing 50 ml of *water* and titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

Acetic Acid, Lead-free

Acetic acid which complies with following additional test:

Boil 25 ml until the volume is reduced to about 15 ml, cool, make alkaline with *lead-free ammonia solution*, add 1 ml of *lead-free potassium cyanide solution*, dilute to 50 ml with *water*, and add 2 drops of *sodium sulphide solution*; no darkening is produced.

Acetic Anhydride : $(CH_3.CO)_2O$ = 102.09

Contains not less than 95.0 per cent of $C_4H_6O_3$.

DESCRIPTION – Clear, colourless liquid with a pungent odour.

DISTILLATION RANGE – Not less than 95 per cent distils between 138° and 141.5°, Appendix 5.3.

CHLORIDE – 3.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 4 g complies with the *limit test for sulphates*, Appendix 3.2.8.

RESIDUE ON IGNITION – Evaporate 5 g to dryness and ignite gently; the residue weighs not more than 1 mg.

ASSAY – Weigh accurately about 2 g and dissolve in 50 ml of *N sodium hydroxide* contained in a stoppered flask, and allow to stand for one hour; titrate the excess of alkali with *N hydrochloric acid*, using *phenolphthalein solution* as indicator. Calculate the number of ml of *N sodium hydroxide* required for 1 g; call this quantity 'a'.

Weigh accurately about 2 g and dissolve in 20 ml of dry *benzene* in a stoppered flask, cool in ice, and add a cold solution of 10 ml of dry *aniline* in 20 ml of dry *benzene*, allow the mixture to stand for one hour, add 50 ml of *N sodium hydroxide* and shake vigorously; titrate the excess of alkali with *N hydrochloric acid*, using *phenolphthalein solution* as indicator. Calculate the number of ml of *N sodium hydroxide* required for 1 g; call this quantity 'b' Calculate the percentage of $C_4H_6O_3$ from the equation $(a - b) \times 10.2$ = percentage of $C_4H_6O_3$.

Acetic Anhydride-dioxan Solution

Add 1 ml of *acetic anhydride* to 50 ml of *dioxan*.

Acetic Bromine Solution

Dissolve 100 g of *potassium acetate* in *glacial acetic acid* and add 4 ml of *bromine* and sufficient *glacial acetic acid* to produce 1000 ml.

Acetone : Propan-2-one; $(CH_3)_2CO$ = 58.08

DESCRIPTION – Clear, colourless, mobile and volatile liquid; taste, pungent and sweetish; odour, characteristic; flammable.

SOLUBILITY – Miscible with *water*, with *alcohol*, with *solvent ether*, and with *chloroform*, forming clear solutions.

DISTILLATION RANGE – Not less than 95.0 per cent distils between 55.5° and 57°, Appendix 5.3.

ACIDITY – 10 ml diluted with 10 ml of freshly boiled and cooled *water*; does not require for neutralisation more than 0.2 ml of *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator.

ALKALINITY – 10 ml diluted with 10 ml of freshly boiled and cooled *water*, is not alkaline to *litmus solution*.

OXIDISABLE SUBSTANCES – To 20 ml add 0.1 ml of *0.1N potassium permanganate*, and allow to stand for fifteen minutes; the solution is not completely decolorised.

METHYL ALCOHOL – Dilute 10 ml with *water* to 100 ml. To 1 ml of the solution add 1 ml of *water* and 2 ml of *potassium permanganate* and *phosphoric acid solution*. Allow to stand for ten minutes and add 2 ml of *oxalic acid* and *sulphuric acid solution*; to the colourless solution add 5 ml of *decolorised magenta solution* and set aside for thirty minutes between 15° and 30°; no colour is produced.

WATER – Shake 10 ml with 40 ml of *carbon disulphide*; a clear solution is produced.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

Acetone, Dry

Acetone which contains not more than 0.3 per cent w/v of water, as determined by the following method:

WATER – Dilute about 12 ml of *acetyl chloride* to 100 ml with *toluene*. Transfer 10 ml to a dry stoppered 250 ml flask, preferably using a pipette with an automatic suction device, add 2 ml, accurately measured, of *pyridine* and immediately stopper tightly and shake vigorously, avoiding wetting the stopper. Add 50 ml, accurately measured, of the sample of acetone and shake vigorously, avoiding wetting the stopper. Allow to stand for five minutes at room temperature, add, by pipette, 1.5 ml of *ethyl alcohol*, shake vigorously, and allow to stand for ten minutes. Add 25 ml of *ethyl alcohol* and titrate with *N sodium hydroxide*, using *1-naphtholphthalein solution* as indicator. Add a slight excess of *N sodium hydroxide* and titrate with *N hydrochloric acid* after adding a few more drops of the indicator. Repeat the experiment with the same quantity of the same reagents in the same manner, omitting acetone. The difference between the two titrations represents the amount of *N sodium hydroxide* which is equivalent to the water present. Each ml of *N sodium hydroxide* is equivalent to 0.01802 g of water.

Acetone-dried Ox Brain

Cut into small pieces a fresh ox brain freed from vascular and connective tissue. Place in *acetone* for preliminary dehydration. Pound in a mortar 30 g of this material with successive quantities, each of 75 ml, of *acetone*, until a dry powder remains after filtration. Dry at 37° for two hours until all acetone is removed.

Acetone Solution, Standard

A 0.05 per cent v/v solution of *acetone* in *water*.

Acetyl Chloride : $CH_3{\cdot}CO{\cdot}Cl = 78.50$

Contains not less than 98.0 per cent w/w of $CH_3{\cdot}CO{\cdot}Cl$, determined by both the Assays described below:

DESCRIPTION – Clear, colourless or very slightly yellow liquid.

SULPHATE – Mix 4 ml with 20 ml of *water*, add 20 mg of *sodium bicarbonate* and evaporate to dryness; the residue complies with the *limit test for sulphates*, Appendix 3.2.8.

PHOSPHORUS COMPOUNDS – Carefully treat 1 ml with 1 ml of *water*, add 1 ml of *nitric acid*, boil, cool, dilute with 20 ml of *water*, add 10 ml of *ammonium nitromolybdate solution* and allow to stand at about 40° for two hours; no yellow precipitate is produced.

ASSAY – *For acidity*—Weigh accurately about 1 g and dissolve in 50 ml of *N sodium hydroxide* and titrate with *N sulphuric acid*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.03925 g of $CH_3{\cdot}CO{\cdot}Cl$.

For chloride—Dilute the neutralised liquid to 250 ml with *water*, mix, and titrate 50 ml with *0.1N silver nitrate*, using *potassium chromate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.007850 g of $CH_3{\cdot}CO{\cdot}Cl$.

Acetylacetone : Pentane-2,4-dione; $CH_3CO{\cdot}CH_2{\cdot}CO{\cdot}CH_3 = 100.11$

DESCRIPTION – A colourless or slightly yellow, flammable liquid; odour, aromatic.

SOLUBILITY – Freely soluble in *water*, miscible with *alcohol*, with *chloroform*, with *solvent ether*, with *acetone* and with *glacial acetic acid*.

BOILING RANGE – Between 138° and 140°, Appendix 5.3.

REFRACTIVE INDEX : At 20°, between 1.452 and 1.453, Appendix 5.14.

Acetylacetone Solution

Add 0.2 ml of *acetylacetone* to 100 ml of *ammonium acetate solution*.

Adenine Sulphate : $(C_5H_5N_5)_2,H_2SO_4,2H_2O = 404.36$

DESCRIPTION – White crystals or crystalline powder.

SOLUBILITY – Slightly soluble in *water*, less soluble in *alcohol*; soluble in solutions of *sodium hydroxide*.

MELTING POINT – About 200°, with decomposition, Appendix 5.11, determined on material dried at 110°.

SULPHATED ASH – Not more than 0.01 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 10.0 per cent, determined by drying in an oven at 105°, Appendix 5.8.

Adrenaline Acid Tartrate : Adrenaline Bitartrate

Of the Indian Pharmacopoeia.

Adrenaline Acid Tartrate, Noradrenaline-free

Adrenaline acid tartrate which complies with the following additional test:

NORADRENALINE – Prepare a chromatogram by *descending paper chromatography*, Appendix 5.4.2. Use, as the stationary phase, the lower layer obtained by shaking together four volumes of *n-butyl alcohol*, one volume of *glacial acetic acid*, and five volumes of *water* and allowing to separate, and, as the mobile phase, the upper layer so obtained. Apply to the paper 0.02 ml of *water* containing 0.1 mg of the adrenaline acid tartrate. Elute for twenty-four hours and spray the dried paper with a freshly prepared 0.44 per cent w/v solution of *potassium ferricyanide* in *buffer solution pH 8.0*; the paper shows only one spot, which is pink.

Agar

Dried gelatinous substance, obtained from *Gelidium*

amansii Lamouronx, *G. cartilagineum* (Linn.) Gaill., *G. pristoides* (Turn.) Kiitz, *Gracilaria confervoides* (Linn.) Greville, *Pterocladia lucida* (Turn.) J.Ag., *P. capillacea* (Gmel.) Born & Thur, and other closely allied members of Rhodophyceae.

DESCRIPTION – Slender, translucent, nearly colourless, lustrous strips about 4 mm wide; or flattened, yellowish bands 4 cm wide; or sheets about 45 to 60 cm long and 10 to 15 cm wide; or greyish-white flakes or coarse powder; odourless or with a slight-odour; taste, mucilaginous.

SOLUBILITY – Practically insoluble in cold *water*, but swells to a gelatinous mass; soluble in boiling *water*.

ACID-INSOLUBLE ASH – Not more than 1.0 per cent, Appendix 3.3.22.

FOREIGN ORGANIC MATTER – Not more than 1.0 per cent, Appendix 3.3.22.

FOREIGN STARCH – Boil 0.1 g with 100 ml of *water*, cool, and add *iodine solution*; no blue colour is produced.

FOREIGN INSOLUBLE MATTER – To 7.5 g add 492.5 g of *water*, boil for fifteen minutes and add *water* to make 500 g, mix well, and to 100 g of the mixture add *water* to make 200 ml, heat to boiling, filter while hot through a tared filtering crucible, rinse the container several times with hot *water* and filter the rinsings through the crucible. Dry the crucible to constant weight at 105°; the residue is not more than 1.0 per cent of the weight taken.

SULPHATED ASH : Not more than 5.0 per cent, Appendix 3.2.7.

LOSS ON DRYING – Loses not more than 18.0 per cent of its weight when dried to constant weight at 105°, Appendix 5.8.

Alcohol

Of the Indian Pharmacopoeia.

Alcohol (90 per cent)

Dilute 947 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.832 to 0.835, Appendix 5.19.

Alcohol (80 per cent)

Dilute 842 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.863 to 0.865, Appendix 5.19.

Alcohol (75 per cent)

Dilute 789 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY : At 15.56°/15.56°, 0.8770 to 0.8772, Appendix 5.19.

Alcohol (70 per cent)

Dilute 737 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.889 to 0.891, Appendix 5.19.

Alcohol (60 per cent) : Dilute 632 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.913 to 0.914, Appendix 5.19.

Alcohol (50 per cent)

Dilute 526 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.934 to 0.935, Appendix 5.19.

Alcohol (40 per cent)

Dilute 421 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.9516 to 0.9518, Appendix 5.19.

Alcohol (25 per cent)

Dilute 263 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.9705 to 0.9713, Appendix 5.19.

Alcohol (20 per cent)

Dilute 210 ml of *alcohol* to 1000 ml with *water*.

SPECIFIC GRAVITY – At 15.56°/15.56°, 0.9755 to 0.9765, Appendix 5.19.

Alcohol, Aldehyde-free

Alcohol which complies with the following additional test:

ALDEHYDE – To 25 ml, contained in a 300-ml flask, add 75 ml of *dinitrophenylhydrazine solution*, heat on a water-bath under a reflux condenser for twenty-four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of *sulphuric acid*, and set aside for twenty-four hours; no crystals are produced.

Alcohol, Sulphate-free

Alcohol which complies with the following additional test:

SULPHATE – 25 ml, evaporated to a volume of about 2 ml, complies with the *limit test for sulphates*, Appendix 3.2.8, a mixture of 2 ml of *dilute hydrochloric acid* and 43 ml of *water*, to which 5 ml of *barium sulphate reagent* has been added, being used as the standard turbidity.

Alizarin Fluorine Blue : 3-Aminomethylalizarin-*NN*-diacetic acid; $C_{19}H_{15}NO_8 = 385.33$

DESCRIPTION – Yellow ochre to orange-brown powder.

SOLUBILITY – Soluble in *dilute ammonia solution*.

SENSITIVITY TO FLUORIDE IONS – Dissolve 0.1 g in two drops of *strong ammonia solution* and two drops of *ammonium acetate solution* and dilute to 20 ml with *water*. To 10 ml of this solution, add 25 ml of the buffer solution described below and dilute to 100 ml with *water*. To one drop of this solution, on a white spot plate, add one drop of a 0.00088 per cent w/v solution of *sodium fluoride* and one drop of a 0.0434 per cent w/v solution of *cerous nitrate* and mix immediately. When viewed in diffused daylight after one minute, the colour appears blue by comparison with the red spot produced when the test is repeated replacing one drop of the solution of sodium fluoride with one drop of *water*.

BUFFER SOLUTION – Dissolve 10.5 g of *sodium acetate* in a mixture of 10 ml of *glacial acetic acid* and 70 ml of *water* and add sufficient *water* to produce 100 ml.

Alizarin Fluorine Blue Solution

Dissolve 0.38 g of *alizarin fluorine blue* in a solution of 0.3 g of *sodium hydroxide* in 25 ml of *water*. Dilute with *water* to about 1500 ml and add 0.5 g of *sodium acetate* and *dilute hydrochloric acid* until a thin layer of the solution is pale pink in colour. Add sufficient *water* to produce 2000 ml.

Alizarin Red S : CI 58005; Sodium Alizarin Sulphonate; Mordant Red 3; $C_{14}H_7NaO_7S,H_2O = 360.27$

DESCRIPTION – Yellowish-brown or orange-yellow powder.

It complies with the following test:

SENSITIVITY – To 5.0 ml of *0.1N sulphuric acid* add 5 ml of *water*, 50 ml of *acetate buffer pH 3.7* and 0.5 ml of a 0.1 per cent w/v solution of the alizarin red S. Add dropwise *0.05M barium perchlorate*; the colour changes from yellow to orange-red.

Alizarin Red S Solution

A 0.1 per cent w/v solution of *alizarin red S*.

Alum : Potash Alum; Aluminium Potassium Sulphate; $KAl(SO_4)_2,12H_2O = 474.40$

Contains not less than 99.5 per cent of $KAl(SO_4)_2,12H_2O$.

DESCRIPTION – Colourless, transparent, crystalline masses or granular crystals or a white powder; odourless; taste, sweetish and astringent.

SOLUBILITY – Very soluble in *water*; practically insoluble in *alcohol*; freely soluble in *glycerin*.

ARSENIC – Not more than 4 parts per million, Appendix 3.2.1.

HEAVY METALS – Dissolve 0.5 g in 10 ml of *water*, add 0.25 ml of *acetic acid* and add *water* to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 3.2.4.

IRON – Dissolve 0.2 g in 20 ml of *water*; 10 ml of the solution complies with the *limit test for iron*, Appendix 3.2.5.

ZINC – Dissolve 1 g in 20 ml of *water*, add 0.5 ml of *dilute hydrochloric acid* and 2 g of *ammonium chloride*, dilute to 50 ml with *water*, add 1 ml of *potassium ferrocyanide solution*, and set aside for five minutes; any opalescence produced is not greater than in a control made by adding 0.5 ml of *dilute hydrochloric acid*, 2 g of *ammonium chloride*, 1 ml of *potassium ferrocyanide solution* to 4 ml of 0.011 per cent w/v solution of *zinc sulphate*, diluting the volume to 50 ml with *water* and setting aside for five minutes.

AMMONIUM SALTS – Dissolve 1 g in 1000 ml of *ammonia-free water*; to 10 ml of the solution add 40 ml of *ammonia-free water* and 2 ml of *alkaline potassium mercuri-iodide solution*; any colour produced is not deeper than in a control made by adding 2 ml of *alkaline potassium mercuri-iodide solution* to 1 ml of *dilute ammonium chloride solution (Nessler's)* in 50 ml of *ammonia-free water*.

ASSAY – Dissolve about 1.7 g, accurately weighed, in sufficient *water* to produce 100 ml. To 20 ml add 25 ml of *0.05M disodium ethylenediaminetetraacetate* and 100 ml of *water*, heat on water-bath for ten minutes, cool, add 5 g of *hexamine*, and titrate with *0.05M lead nitrate*, using 0.4 ml of *xylenol orange solution* as indicator. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.02372 g of $KAl(SO_4)_2,12H_2O$.

Alumina, Anhydrous

An aluminium oxide, dehydrated and activated by heat treatment and consisting of γ-Al_2O_3. The particle size is such that all the aluminium oxide passes through *No. 150 sieve* but is retained on *No. 75 sieve*.

Aluminium Ammonium Sulphate : Ammonia Alum, $AlNH_4(SO_4)_2,12H_2O = 453.32$

DESCRIPTION – Large, colourless crystals or crystalline fragments or a white powder.

SOLUBILITY – Freely soluble in *water*; insoluble in *alcohol*; highly soluble in boiling *water*.

Aluminium Chloride, Anhydrous : $AlCl_3 = 133.34$

Contains not less than 98.0 per cent of $AlCl_3$.

DESCRIPTION – White, grey or yellow crystalline powder, or crystalline masses, fuming in air, with a strong odour of hydrochloric acid.

SOLUBILITY – Freely soluble in *water*, with evolution of heat; freely soluble in *nitrobenzene*. The solutions produced are clear.

ASSAY – Weigh accurately about 0.5 g and dissolve in sufficient *water* to produce 250 ml. To 50 ml of this solution add 5 ml of *dilute nitric acid*, 50 ml of *0.1N silver nitrate* and 5 ml of *nitrobenzene* and shake well. Add 5 ml of *ferric ammonium sulphate solution* and titrate with *0.1N ammonium thiocyanate* until a reddish-brown colour is produced that persists for five minutes. Each ml of *0.1N silver nitrate* is equivalent to 0.004445 g of $AlCl_3$.

Aluminium Chloride Solution

Dissolve 35.9 g of *anhydrous aluminium chloride* in sufficient *water* to produce 100 ml, add 0.5 g of *decolorising charcoal*, stir for ten minutes, filter and add to the filtrate with continuous stirring sufficient of a 10 per cent w/v solution of *sodium hydroxide* to adjust the pH to about 1.5.

NOTE – Care should be taken in dissolving anhydrous aluminium chloride in water.

Amaranth S : CI 16185; Acid Red 27, $C_{20}H_{10}N_2Na_3O_{10}S_3$ = 604.46

DESCRIPTION – A deep brown or deep red-brown, fine powder.

Complies with the following test:

HOMOGENEITY – Carry out the test for separating power described under *cellulose MN 300*, applying to the plate a solution containing only the amaranth S; the chromatogram shows only one spot at an R_f of about 0.6. When used for the titration of iodine and iodides with potassium iodate, the colour changes from orange-red to yellow.

Aminoacetic acid : Glycine; $NH_2{\cdot}CH_2{\cdot}COOH$ = 75.07

Contains not less than 99.0 per cent of $C_2N_5NO_2$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*; practically insoluble in *ethyl alcohol* and in *solvent ether*.

ACIDITY OR ALKALINITY – Dissolve 1 g in 100 ml of *carbon dioxide-free water*. To 50 ml add 0.1 ml of *0.1N sodium hydroxide*; the pH of the solution is not more than 6.5. To the other 50 ml add 0.1 ml of *0.1N hydrochloric acid*; the pH of the solution is not more than 5.5, Appendix 5.10.

AMMONIA – Dissolve 1 g in 45 ml of *ammonia-free water*, add 5 ml of *sodium hydroxide solution*, and distil 15 ml into about 20 ml of *ammonia-free water*. Adjust the volume to 50 ml with *ammonia-free water* and add 2 ml of *alkaline potassium mercuri-iodide solution*. The colour of the resulting solution is not deeper than that produced by adding 2 ml of *alkaline potassium mercuri-iodide solution* to a mixture of 45 ml of *ammonia-free water* and 5 ml of *dilute ammonium chloride solution (Nessler's)*.

IRON AND HEAVY METALS – Dissolve 1 g in 45 ml of *water*, add 5 ml of *dilute ammonia solution*, and pass *hydrogen sulphide* through the solution for a few seconds; not more than a slight darkening is produced.

CHLORIDE – Dissolve 1 g in 50 ml of *water*, and add 1 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; no opalescence is produced.

SULPHATE – Dissolve 2 g in 50 ml of *water*, add 1 ml of *dilute hydrochloric acid* and 1 ml of *barium chloride solution* and allow to stand for one hour; no turbidity or precipitate is produced.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Carry out the method for the *determination of nitrogen*, Appendix 3.3.5, using about 0.6 g accurately weighed and 8 ml of *nitrogen-free sulphuric acid*. Each ml of *0.1N sulphuric acid* is equivalent to 0.007507 g of $C_2H_5NO_2$.

(+)-2-Aminobutanol : (+)-2-Aminobutan-1-ol $CH_3.CH_2.CH(NH_2).CH_2OH$ = 89.14.

DESCRIPTION – An oily liquid.

WT. PER ML – At 20°, about 0.95 g, Appendix 5.19.

REFRACTIVE INDEX – At 20°, about 1.453, Appendix 5.14.

SPECIFIC OPTICAL ROTATION – At 20°, about +13°, determined with 2 per cent w/v solution in *alcohol*, Appendix 5.12.

4-Amino-3-hydroxynaphthalene-1-sulphonic Acid: 1-Amino-2-naphthol-4-sulphonic acid; $C_{10}H_9NO_4S$ = 239.25

DESCRIPTION – Crystalline powder.

MELTING POINT – About 300°, with decomposition, Appendix 5.11.

Aminohydroxynaphthalenesulphonic Acid Solution

Dissolve 0.5 g of *4-amino-3-hydroxynaphthalene-1-sulphonic acid* in about 150 ml of a 15 per cent w/v solution of *sodium metabisulphite*, warming to assist solution, if necessary. Add 5 ml of a 20 per cent w/v solution of *sodium sulphite*, mix, and add sufficient of the sodium metabisulphite solution to produce 200 ml.

3-Aminophenol : *m*-Aminophenol; $NH_2{\cdot}C_6H_4{\cdot}OH$ = 109.13

DESCRIPTION – White or slightly coloured crystals or powder.

SOLUBILITY – Soluble in *water*.

MELTING RANGE – 121° to 123°, Appendix 5.11.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

4-Aminophenol : *p*-Aminophenol; $NH_2 \cdot C_6H_4 \cdot OH$ = 109.13

DESCRIPTION – White or almost white, crystalline powder.

SOLUBILITY – Soluble in *water*.

MELTING POINT – About 184°, with decomposition, Appendix 5.11.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

Aminopyrazolone : Aminophenazone, 4-Aminoantipyrine; 4-Aminophenazone; 4-Amino-2,3-dimethyl-1-phenyl-5-pyrazolone; $C_{11}H_{13}N_3O = 203.24$

DESCRIPTION – Yellow to buff powder or crystals.

SOLUBILITY – Freely soluble in *alcohol*; soluble in *water* and in *solvent ether*.

MELTING RANGE – Between 107° and 109°, Appendix 5.11.

It complies with the following test:

SENSITIVITY – To 2 ml of a 5 per cent w/v solution of *phenol*, add 10 ml of a 4 per cent solution of *borax*, mix, add 1 ml of a 3 per cent w/v solution of the aminopyrazolone and mix; add a further 10 ml of the solution of *borax* and sufficient *water* to produce 50 ml, and allow to stand for ten minutes; *extinction* of the resulting solution at about 505 nm, not less than 0.2, Appendix 5.15 A.

Aminopyrazolone Solution

A 0.1 per cent w/v solution of *aminopyrazolone* in *buffer solution pH 9.0*.

Ammonia, *x*N

Solutions of any normality *x*N may be prepared by diluting 75*x* ml of *strong ammonia solution* to 1000 ml with *water*.

Ammonia-Ammonium Chloride Solution, Strong

Dissolve 67.5 g of *ammonium chloride* in 740 ml of *strong ammonia solution* and add sufficient *water* to produce 1000 ml.

Ammonia Solution, Alcoholic

A solution of ammonia gas in *alcohol*.

DESCRIPTION – Clear, colourless liquid having a strong odour of ammonia.

SPECIFIC GRAVITY – About 0.80, Appendix 5.19.

CONTENT OF AMMONIA (NH_3) – Between 9.0 and 11.0 per cent w/w.

STORAGE – Store in alkali-resistant containers, in cool place.

Ammonia Solution, Dilute

Contains approximately 10 per cent w/w of NH_3.

Dilute 425 ml of *strong ammonia solution* to 1000 ml with *water*.

WT. PER ML – At 25°, about 0.960 g, Appendix 5.19.

STORAGE – Dilute Ammonia Solution should be kept in a well-closed container, in a cool place.

Ammonia Solution, Iron-free

Dilute ammonia solution which complies with the following additional test: Evaporate 5 ml nearly to dryness on a water-bath, add 40 ml of *water*, 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 2 drops of *thioglycolic acid*, mix, make alkaline with *iron-free ammonia solution*, and dilute to 50 ml with *water*; no pink colour is produced.

Ammonia Solution, Lead-free

Dilute ammonia solution which complies with following additional test: To 20 ml add 1 ml of *lead-free potassium cyanide solution*, dilute to 50 ml with *water*, and add 2 drops of *sodium sulphide solution*; no darkening is produced.

Ammonia Solution, Strong

Contains 25.0 per cent w/w of NH_3 (limits, 24.5 to 25.5). About 13.5 N is strength.

DESCRIPTION – Clear, colourless liquid; odour, strongly pungent and characteristic.

SOLUBILITY – Miscible with *water* in all proportions

WT. PER ML – At 25°, about 0.91 g, Appendix 5.19.

ARSENIC – Not more than 0.4 part per million, Appendix 3.2.1.

HEAVY METALS – Evaporate 5 ml to dryness on a water-bath. To the residue, add 1 ml of *dilute hydrochloric acid* and evaporate to dryness. Dissolve the residue in 2 ml of *dilute acetic acid* and add *water* to make 25 ml; the limit of heavy metals is 15 parts per million, Appendix 3.2.4.

IRON – Evaporate 40 ml on a water-bath to about 10 ml. The solution complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – Evaporate 40 ml on a water-bath to about 5 ml. The solution complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Evaporate 20 ml on a water-bath to about 5 ml. The solution complies with the *limit test for sulphates*; Appendix 3.2.8.

TARRY MATTER – Dilute 5 ml with 10 ml of *water*, mix with 6 g of powdered *citric acid* in a small flask, and rotate until dissolved; no tarry or unpleasant odour is perceptible.

NON-VOLATILE RESIDUE – Evaporate 50 ml to dryness in a tared porcelain dish and dry to constant weight at 105°; not more than 5 mg of residue remains.

ASSAY – Weigh accurately about 3 g in flask containing 50 ml of *N sulphuric acid* and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.01703 g of NH_3.

STORAGE – Preserve Strong Ammonia Solution in a well-closed container, in a cool place.

Ammonium Acetate : $CH_3.COO.NH_4 = 77.08$

DESCRIPTION – Colourless crystals or crystalline masses; odour, slightly acetous; very deliquescent.

SOLUBILITY – Very soluble in *water*, and in *alcohol*.

ACIDITY – Dissolve 1 g in 20 ml of *carbon dioxide-free water*; the solution is not more acid than pH 6.5, using *bromothymol blue solution* as indicator.

CHLORIDE – 3.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Dissolve 4.0 g in 5 ml of *water*, add 10 mg of *sodium bicarbonate*, evaporate to dryness, and heat at 120° until the ammonium acetate is volatilised. The residue complies with the *limit test for sulphates*, Appendix 3.2.8.

SULPHATED ASH – Not more than 0.035 per cent, Appendix 3.2.7.

Ammonium Acetate Solution

Dissolve 150 g of *ammonium acetate* in 200 ml of *water*. Add 3 ml of *glacial acetic acid* and dilute to 1000 ml with *water*.

Ammonium Carbonate

A variable mixture of ammonium bicarbonate ($NH_4.HCO_3$) and ammonium carbamate ($NH_2.CO_2.NH_4$). Contains the equivalent of not less than 30.0 per cent of NH_3.

DESCRIPTION – Translucent, hard, crystalline masses; odour, strongly ammoniacal; taste, pungent and ammoniacal. Exposed to air, it partially dissociates and volatilises, and becomes converted into porous lumps or a white powder.

SOLUBILITY – Freely soluble in *water*; partly soluble in *alcohol* yielding a residue of the bicarbonate.

IRON – Boil 2.5 g with *water* until all the ammonia has been driven off; the solution complies with *the limit test for iron*, Appendix 3.2.5.

CHLORIDE – 10 g, boiled with *water* until all the ammonia has been driven off, complies with *the limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 10 g, boiled with *water* until all the ammonia has been driven off, complies with *the limit test for sulphates*, Appendix 3.2.8.

TARRY MATTER – Mix 5 g with 15 ml of *water* and 7 g of *citric acid* and stir until dissolved; no tarry odour is produced.

NON-VOLATILE MATTER – When volatilised at a temperature below red heat leaves not more than 0.025 per cent of residue.

ASSAY – Weigh accurately about 2 g and dissolve in 50 ml of *N sulphuric acid* diluted with 50 ml of *water*, boil, cool, and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.01703 g of NH_3.

STORAGE – Ammonium Carbonate should be kept in a well-closed container.

Ammonium Carbonate Solution

Dissolve 5 g of *ammonium carbonate* in a mixture of 7.5 ml of *dilute ammonia solution* and 50 ml of *water*; add sufficient quantity of *water* to produce 100 ml and filter, if necessary.

Ammonium Chloride

Of the Indian Pharmacopoeia.

Ammonium Chloride, 2M

Dissolve 106.98 g of *ammonium chloride* in sufficient *water* to produce 1000 ml.

Ammonium Chloride Solution

A 10.0 per cent w/v solution of *ammonium chloride* in *water*.

Ammonium Chloride-Ammonium Hydroxide Solution

Mix 1 volume of *water* and 2 volumes of *strong ammonia solution* and saturate with *ammonium chloride*.

Ammonium Chloride Solution (Nessler's)

Dissolve 3.15 g of *ammonium chloride* in sufficient quantity of *ammonia-free water* to produce 1000 ml.

Ammonium Chloride Solution, Dilute (Nessler's)

Mix 10 ml of *ammonium chloride solution (Nessler's)* with sufficient quantity of *ammonia-free water* to produce 1000 ml.

Ammonium Cobaltothiocyanate Solution

Dissolve 37.5 g of *cobalt nitrate* and 150 g of *ammonium thiocyanate* in sufficient *water* to produce 1000 ml.

Ammonium Cobaltothiocyanate Solution should be freshly prepared.

Ammonium Mercurithiocyanate Solution : Mercuric Ammonium Thiocyanate Solution

Dissolve 30 g of *ammonium thiocyanate* and 27 g *mercuric chloride* in sufficient *water* to produce 1000 ml.

Ammonium Molybdate : $(NH_4)_6\ Mo_7\ O_{24},\ 4H_2O$ = 1235.86

Contains about 80 to 83 per cent of MoO_3.

DESCRIPTION – White crystals or crystalline masses, sometimes with a yellowish or greenish tint.

SOLUBILITY – Soluble in *water.*

PHOSPHATE – Dissolve 5.0 g in 5 ml of dilute *ammonia solution* and 15 ml of *water*; the solution is not more than slightly turbid; add it to 25 ml of *nitric acid* and 50 ml of *water* and allow to stand at about 40° for six hours; not more than a slight yellow precipitate is produced.

ASSAY – Weigh accurately about 0.2 g and dissolve in a mixture of 40 ml of *dilute ammonia solution* and 50 ml of *water.* Add 20 ml of *glacial acetic acid*, heat to boiling, and add 10 ml of *lead acetate solution* and 40 ml of *water.* Boil gently until the precipitate becomes granular, collect the precipitate in a Gooch crucible, wash with hot *water*, dry, and ignite at a dull red heat. Each g of the residue is equivalent to 0.3920 g of MoO_3.

Ammonium Molybdate Solution

A 10.0 per cent w/v solution of *ammonium molybdate* in *water.*

Ammonium Molybdate Solution, Dilute

A 3 per cent w/v solution of *ammonium molybdate* in *water.*

Ammonium Nitrate : $NH_4\ NO_3$ = 80.04

DESCRIPTION – Colourless crystals.

SOLUBILITY – Freely soluble in *water.*

ACIDITY – A solution in *water* is slightly acid to *litmus solution.*

CHLORIDE – 3.5 g complies with the *limit test for sulphates*, Appendix 3.2.2.

SULPHATE – 5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

Ammonium Nitromolybdate Solution

To a solution of 125 g of *molybdic acid* in a mixture of 80 ml of *strong ammonia solution* and 320 ml of *water*, add a solution of 400 g of *ammonium nitrate* in sufficient *water* to produce 1000 ml, followed by a mixture of 380 ml of *nitric acid* and 620 ml of *water.* Allow to stand for twenty-four hours at about 35° and filter or decant the clear solution.

NOTE – Ammonium nitromolybdate solution is liable to become insensitive during storage.

Ammonium Oxalate : $(CO_2NH_4)_2,H_2O$ = 142.11

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *water.*

CHLORIDE – 2 g, with an additional 20 ml of *dilute nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Dissolve 1 g in 50 ml of *water*, add 2.5 ml of *hydrochloric acid* and 1 ml of *barium chloride solution* and allow to stand for one hour; no turbidity or precipitate is produced.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

Ammonium Oxalate Solution

A 2.5 per cent w/v solution of *ammonium oxalate* in *water.*

Ammonium Persulphate : Ammonium Peroxodisulphate; $(NH_4)_2S_2O_8$ = 228.20

Contains not less than 98.0 per cent of $(NH_4)_2S_2O_8$.

DESCRIPTION – White granular crystals or crystalline powder.

SOLUBILITY – Soluble in *water.*

CHLORIDE – 5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Dissolve 1 g of *ferrous sulphate* and 6 g of *potassium iodide* in a mixture of 50 ml of *water* and 50 ml of *dilute sulphuric acid.* Add about 0.5 g, accurately

weighed, of the finely powdered sample to half of this solution and allow to stand in a stoppered flask for thirty minutes. Titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Repeat the operation using the remaining portion of the solution and omitting the sample, and subtract the second burette reading from the first. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.01141 g of $(NH_4)_2S_2O_8$.

Ammonium Reineckate : Ammonium Tetrathiocyanatodiamminochromate (III) Monohydrate
$(NH_4[Cr(NH_3)_2(SCN)\ \ H_2O = 354.42$.

Contains not less than 95.0 per cent of $C_4H_{10}N_7S_4Cr,H_2O$.

DESCRIPTION – Red crystals or a crystalline powder.

SOLUBILITY – Sparingly soluble in *water*, giving a clear red solution.

ASSAY – Weigh accurately about 0.2 g and boil with 10 ml of *sodium hydroxide solution* until decomposed and a green precipitate is produced. Cool, add an excess of *dilute nitric acid*, and again cool. Add 25 ml of *0.1N silver nitrate*, and titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.008861 g of $C_4H_{10}N_7S_4Cr,H_2O$.

SENSITIVITY – Dissolve 50 mg in 10 ml of *water*. Add 0.2 ml of the solution to 1 ml of a solution of 10 mg of *choline chloride* in 20 ml of *water* and shake gently; a distinct precipitate forms within five to ten seconds.

Ammonium Reineckate Solution

Shake about 0.2 g of *ammonium reineckate* with 20 ml of *water* frequently during one hour and filter.

NOTE – It should not be used later than forty-eight hours after its preparation.

Ammonium Sulphamate : $NH_2.SO_3NH_4 = 114.12$

Contains not less than 98.0 per cent of $H_6O_3N_2S$.

DESCRIPTION – White crystals or a crystalline powder.

SOLUBILITY – Freely soluble in *water*.

MELTING RANGE – 130° to 133°, Appendix 5.11.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Boil about 1.5 g accurately weighed, with a mixture of 10 ml of *sulphuric acid* and 50 ml of *water* for two hours under a reflux condenser. Transfer to an ammonia distillation apparatus, make alkaline with a 30 per cent w/v solution of *sodium hydroxide*, and distil, collecting the distillate in 50 ml of *N sulphuric acid*. Titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.05706 g of $H_6O_3N_2S$.

Ammonium Sulphate : $(NH_4)_2SO_4 = 132.13$

DESCRIPTION – Colourless crystals or white granules.

SOLUBILITY – Very soluble in *water*; insoluble in *alcohol*.

ACIDITY – 1 g dissolved in 10 ml of *carbon dioxide-free water*; has a pH of 5.0 to 6.0, Appendix 5.10.

CHLORIDE – 3.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Ammonium Sulphide Solution

Saturate 120 ml of *dilute ammonia solution* with washed *hydrogen sulphide*, and add 80 ml of *dilute ammonia solution*.

NOTE – Ammonium sulphide solution must be recently prepared.

Ammonium Thiocyanate : $NH_4SCN = 76.12$

DESCRIPTION – Colourless crystals.

SOLUBILITY – Very soluble in *water*, forming a clear solution; freely soluble in *alcohol*.

CHLORIDE – Dissolve 1 g in 30 ml of *hydrogen peroxide solution*, add 1 g of *sodium hydroxide*, warm gently, rotate the flask until a vigorous reaction commences and allow to stand until the reaction is complete; add a further 30 ml of *hydrogen peroxide solution*, boil for two minutes, cool, and add 10 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; any opalescence produced is not greater than that obtained by treating 0.2 ml of *0.01N hydrochloric acid* in the same manner.

SULPHATED ASH – Moisten 1 g with *sulphuric acid*, and ignite gently, again moisten with *sulphuric acid* and ignite; the residue weighs not more than 2.0 mg.

Ammonium Thiocyanate Solution

A 10.0 per cent w/v solution of *ammonium thiocyanate* in *water*.

Ammonium Vanadate : $NH_4VO_3 = 116.98$.

Contains not less than 98.0 per cent of NH_4VO_3.

DESCRIPTION – White, crystalline powder.

ASSAY – Accurately weigh about 0.5 g and dissolve in 30 ml of *water* and 1 ml of *sulphuric acid*, and pass *sulphur dioxide* into the solution until reduction is complete. Remove the excess of sulphur dioxide by boiling gently in

a current of carbon dioxide, cool and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.0117 g of NH_4VO_3.

Amyl Acetate : $CH_3.CO.O.C_5H_{11} = 130.19$

Contains not less than 97.0 per cent of $C_7H_{14}O_2$.

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

SOLUBILITY – 1 ml dissolves in a mixture of 10 ml of *alcohol* and 10 ml of *water*, yielding a clear solution, which becomes turbid on the addition of 2 ml of *water*.

ACIDITY – 1 g, dissolved in 10 ml of *alcohol*, requires the addition of not more than 0.1 ml of *0.1N alcoholic potassium hydroxide* to give a pink colour with *phenolphthalein solution*.

DISTILLATION RANGE – Not less than 90 per cent distils between 136° and 142°, Appendix 5.3.

WT. PER ML – At 25°, about 0.87 g, Appendix 5.19.

MOISTURE – Miscible with an equal volume of *carbon disulphide*, and of *benzene*, yielding clear solutions.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

ASSAY – Weigh accurately about 2 g and dissolve in 25 ml of *alcohol*, add 25 ml of *N alcoholic potassium hydroxide*, boil under a reflux condenser for one hour and titrate the excess of alkali with *N hydrochloric acid*, using *phenolphthalein solution* as indicator. Each ml of *N alcoholic potassium hydroxide* is equivalent to 0.1302 g of $C_7H_{14}O_2$.

Amyl Alcohol : $C_5H_{11}OH = 88.15$

Consists principally of 3-methylbutan-1-ol with a small proportion of 2-methylbutan-1-ol.

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

SOLUBILITY – Slightly soluble in *water*; miscible with most organic solvents.

DISTILLATION RANGE – Not less than 98 per cent distils between 125° and 135°, Appendix 5.3.

WT. PER ML – At 25°, about 0.81 g, Appendix 5.19.

Aniline : $C_6H_5NH_2 = 93.13$

DESCRIPTION – Colourless or pale yellow, oily liquid, readily discolouring on exposure to air and light.

DISTILLATION RANGE – Not less than 95 per cent distils between 182° and 184°, Appendix 5.3.

WT. PER ML – At 25°, about 1.02 g, Appendix 5.19.

ACID-INSOLUBLE MATTER – Dissolve 5 ml in 25 ml of *dilute hydrochloric acid*, the solution is clear or not more than slightly turbid.

Aniline Blue, Water-soluble

A water-soluble dye consisting of a mixture of the tri sulphonates of triphenylpararosaniline and of diphenylpararosaniline.

Anisaldehyde : 4-Methoxybenzaldehyde
$CH_3O.C_6H_4.CHO = 136.14$.

DESCRIPTION – Oily liquid; odour, characteristic.

SOLUBILITY – Slightly soluble in *water*; miscible with *alcohol* and with *solvent ether*.

BOILING POINT – About 248°, Appendix 5.3.

Anisaldehyde Solution

Mix, in order, 0.5 ml of *anisaldehyde*, 10 ml of *glacial acetic acid*, 85 ml of *methanol* and 5 ml of *sulphuric acid*.

Anthrone : $C_6H_4.CO.C_6H_4.CH_2 = 194.23$

DESCRIPTION – Pale yellow, crystalline powder.

SOLUBILITY – Soluble in *benzene*, and in *sulphuric acid*.

MELTING RANGE – 153° to 157°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

SENSITIVITY – Add carefully 6 ml of a 0.2 per cent w/v solution in a mixture of 19 ml of *sulphuric acid* and 1 ml of *water* to 3 ml of *water* containing 15 µg of *dextrose*. Heat in a water-bath for five minutes, taking precautions against loss of water by evaporation. The solution is darker green than is a solution prepared in a similar manner but omitting the dextrose.

Antimony Trichloride : $SbCl_3 = 228.11$

Contains not less than 97.0 per cent $SbCl_3$.

DESCRIPTION – Colourless crystals, fuming in moist air.

SOLUBILITY – Very soluble in *ethyl alcohol* and in *chloroform*, forming solutions which are not more than slightly turbid.

ASSAY – Weigh accurately about 0.5 g, dissolve in 30 ml of *water* containing 4 g of *sodium potassium tartrate*, add 2 g of *sodium bicarbonate* and titrate with *0.1N iodine*. Each ml of *0.1N iodine* is equivalent to 0.01141 g of $SbCl_3$.

Antimony Trichloride Solution

To 100 ml of a 22 per cent w/v solution of *antimony tri-*

chloride in *alcohol-free chloroform*, add 2.5 ml of *acetyl chloride*, and allow to stand for half an hour

Antimony Trichloride Solution in Ethylene Chloride

Prepare a 22.0 per cent w/v solution of *antimony trichloride* in dry *ethylene chloride* which has been purified by passing it down a column of *silica gel*, and allow the solution to stand for twenty-four hours; to 100 ml add 2.5 ml of *acetyl chloride* and allow to stand for a further twenty-four hours before use.

Arachidic Alcohol : Eicosan-1-ol; $CH_3.(CH_2)_{18}.CH_2OH$ = 298.55

DESCRIPTION – Colourless, waxy or crystalline solid.

Material containing over 95 per cent of $C_{20}H_{42}O$ is commercially available.

Arachis Oil

Of the Indian Pharmacopoeia.

Arsenic Trioxide : As_2O_3 = 197.82

Contains not less than 99.8 per cent of As_2O_3.

DESCRIPTION – Heavy, white powder.

SOLUBILITY – Sparingly soluble in *water*; more readily soluble in *water* on the addition of *hydrochloric acid*, or solutions of alkali hydroxides or carbonates.

ARSENIOUS SULPHIDE – Weigh accurately 0.50 g and dissolve in 10 ml of *dilute ammonia solution*; forms a clear colourless solution which, when diluted with an equal volume of *water* and acidified with *hydrochloric acid*, does not become yellow.

NON-VOLATILE MATTER – Leaves not more than 0.1 per cent of residue when volatilised.

ASSAY – Weigh accurately about 0.2 g and dissolve in 20 ml of boiling *water* and 5 ml of *N sodium hydroxide*, cool, add 5 ml of *N hydrochloric acid* and 3 g of *sodium bicarbonate*, and titrate with *0.1N iodine*. Each ml of *0.1N iodine* is equivalent to 0.004946 g of As_2O_3.

Arsenomolybdate Solution

Dissolve 25 g of *ammonium molybdate* in about 450 ml of *water*, add 21 ml of *sulphuric acid*, mix, add 3 g of *disodium hydrogen arsenate* dissolved in 25 ml of water. Mix and place in an incubator at 37° for twenty-four to forty-eight hours.

STORAGE – Store in a glass-stoppered bottle.

Ascorbic Acid : L-Ascorbic Acid

Of the Indian Pharmacopoeia.

Atropine Sulphate

Of the Indian Pharmacopoeia.

Barbitone Sodium : Sodium 5,5-Diethylbarbiturate; Barbital Sodium; $C_8H_{11}N_2NaO_3$ = 206.19

Contains not less than 98.5 per cent of $C_8H_{11}N_2NaO_3$, calculated with reference to the dried substance.

DESCRIPTION – White, crystalline powder; odourless, taste, bitter.

SOLUBILITY – Freely soluble in *water*; slightly soluble in *alcohol*; insoluble in *chloroform* and in *solvent ether*.

ALKALINITY – A 5 per cent w/v solution is alkaline to *phenolphthalein solution*.

FREE BARBITONE – Shake 0.5 g with 20 ml of *solvent ether* for 10 minutes; filter, evaporate the ether in a tared dish, and dry the residue at 105° for one hour; the residue weighs not more than 3 mg.

LOSS ON DRYING – Loses not more than 1.0 per cent of its weight when dried to constant weight at 105°, Appendix 5.8.

ASSAY – Weigh accurately about 0.5 g and dissolve in 5 ml of *water*, add 2 ml of *dilute hydrochloric acid*, and extract with successive quantities, each of 25 ml, of a mixture of seven volumes of *chloroform*, two volumes of *ethyl alcohol*, and one volume of *solvent ether* until complete extraction is obtained. Wash the combined extracts with 2 ml of *water* containing 0.05 ml of *dilute hydrochloric acid*, remove the solvent, and dry the residue to constant weight at 105°. Each g of residue is equivalent to 1.119 g of $C_8H_{11}N_2NaO_3$.

Barbitone Sodium, 0.1M

Dissolve 20.62 g of *barbitone sodium* in sufficient *water* to produce 1000 ml.

Barium Chloride : $BaCl_2,2H_2O$ = 244.27

DESCRIPTION – Colourless crystals.

SOLUBILITY – Freely soluble in *water*.

LEAD – Dissolve 1 g in 40 ml of recently boiled and cooled *water*, add 5 ml of *lead-free acetic acid*, render alkaline with *lead-free ammonia solution* and add 2 drops of *lead-free sodium sulphide solution*; not more than a slight colour is produced.

NITRATE – Dissolve 1 g in 10 ml of *water*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid* and heat to boiling; the blue colour does not entirely disappear.

Barium Chloride Solution

A 10.0 per cent w/v solution of *barium chloride* in *water*.

Barium Hydroxide : $Ba(OH)_2,8H_2O = 315.47$

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *water*, yielding a slightly turbid solution.

LEAD – Dissolve 0.5 g in 5 ml of *lead-free acetic acid* and 40 ml of *water*, boil gently to remove carbon dioxide, cool, render alkaline with *lead-free ammonia solution* and add 2 drops of *lead-free sodium sulphide solution*; not more than a slight colour is produced.

CARBONATE – Dissolve 1 g in 25 ml of recently boiled and cooled *water*; the solution is not more than slightly turbid, and becomes clear on the addition of 1 ml of *hydrochloric acid*.

CHLORIDE – 3 g, with an additional 2 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

NITRATE – Dissolve 0.5 g in 10 ml of *dilute acetic acid*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the blue colour does not entirely disappear.

Barium Hydroxide Solution

A 3.0 per cent w/v solution of *barium hydroxide* in *water*.

Barium Perchlorate : $Ba(ClO_4)_2 = 336.27$

DESCRIPTION – White powder.

SOLUBILITY – Soluble in *water*.

CHLORIDE – 1.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

CHLORATE – 1.0 g, dissolved in 40 ml of *water* containing 2 ml of *nitric acid* and 0.1 g of *sodium nitrate* and boiled for ten minutes, complies with the *limit test for chlorides*, Appendix 3.2.2.

Barium Sulphate

Of the Indian Pharmacopoeia.

Benzaldehyde : $C_6H_5.CHO = 106.12$

DESCRIPTION – Colourless to pale yellow, oily liquid with an odour of bitter almonds.

DISTILLATION RANGE – Not less than 95 per cent distils between 177° and 180°, Appendix 5.3.

WT. PER ML – At 20°, 1.044 to 1.047 g, Appendix 5.19.

REFRACTIVE INDEX – At 20°, 1.544 to 1.546, Appendix 5.14.

SULPHATED ASH – Not more than 0.01 per cent, Appendix 3.2.7.

Benzalkonium Chloride Solution

Of the Indian Pharmacopoeia.

Benzene : $C_6H_6 = 78.11$

DESCRIPTION – Colourless, transparent liquid; flammable.

DISTILLATION RANGE – Not less than 95 per cent distils between 79.5° and 81°, Appendix 5.3.

WT. PER ML – At 20°, 0.876 to 0.881 g, Appendix 5.19.

SULPHUR COMPOUNDS – Boil 10 ml with 1 ml of *ethyl alcohol* and 3 ml of *potassium plumbite solution* for fifteen minutes under a reflux condenser, and allow to stand for five minutes; the aqueous layer remains colourless.

THIOPHENE – Shake 2 ml with 15 ml of *sulphuric acid* containing 3 mg of *isatin* in a stoppered tube for five minutes and allow to separate; no blue or green colour is produced.

NON-VOLATILE METTER – Leaves not more than 0.01 per cent w/v of residue, when evaporated on a water-bath and dried to constant weight at 105°.

Benzethonium Chloride : Benzyldimethyl-2-[2-[4-(1,1, 3, 3 - tetramethylbutyl) phenoxy]ethoxy Ethylammonium Chloride Monohydrate; $C_{27}H_{42}ClNO_2,H_2O = 466.12$

DESCRIPTION – White crystals, having a mild odour.

SOLUBILITY – Soluble in *water*, in *alcohol*, and in *chloroform*; slightly soluble in *solvent ether*.

MELTING RANGE – Between 158° and 163°, on dried sample, Appendix 5.11.

LOSS ON DRYING – Loses not more than 5.0 per cent of its weight when dried at 105° for four hours, Appendix 5.8.

AMMONIUM COMPOUNDS – To 5 ml of a solution (1 in 50) add 3 ml of *N sodium hydroxide*, heat to boiling the odour of the ammonia is not perceptible.

STORAGE – Store in well-closed containers, protected from light.

Benzidine : $4,4'\text{-}NH_2.C_6H_4.C_6H_4NH_2 = 184.23$.

DESCRIPTION – Pale buff, crystalline powder.

SOLUBILITY – Freely soluble in *alcohol (90 per cent)* yielding a clear solution; 1 g dissolves in a mixture of 3 ml of *dilute hydrochloric acid* and 25 ml of *water*, yielding a clear solution.

MELTING RANGE – 128° to 129°, Appendix 5.11.

ORGANIC IMPURITIES – Dissolve 0.1 g in 5 ml of *glacial acetic acid*; the solution is clear and not more than faintly coloured; add 5 ml of a mixture of equal volumes of *hydrogen peroxide solution* and *water*; no darkening is produced.

SENSITIVITY – To 2 ml of the solution from the test for organic impurities, add 1 ml of a freshly prepared 0.0001 per cent solution of blood in *water*; a blue or greenish blue colour is produced.

Benzoic Acid

Of the Indian Pharmacopoeia.

p-Benzoquinone : (1,4-Benzoquinone); $C_6H_4O_2$ = 108.10

DESCRIPTION – Yellow, crystalline powder.

SOLUBILITY – Slightly soluble in *water*.

MELTING RANGE – 113° to 116°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Benzoyl Chloride : $C_6H_5{\cdot}CO{\cdot}Cl$ = 140.57

Contains not less than 97.0 per cent of C_7H_5OCl.

DESCRIPTION – Pale yellowish liquid, fuming in moist air.

SOLUBILITY – Practically insoluble in *water*; soluble in most organic solvents; slowly and almost completely soluble in *sodium hydroxide solution*.

ASSAY – Dissolve about 2 g, weighed accurately, in 50 ml of *N sodium hydroxide* and titrate with *N sulphuric acid*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.07029 g of C_7H_5OCl.

Benzyl Alcohol

Of the Indian Pharmacopoeia.

Benzyl Benzoate

Of the Indian Pharmacopoeia.

Benzyl Penicillin Sodium

Benzyl Penicillin (Sodium) of the Indian Pharmacopoeia.

Biotin : $C_{10}H_{16}N_2O_3S$ = 244.31

DESCRIPTION – White, crystalline powder.

MELTING RANGE – 230° to 232°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – At 22°, +92°, determined in a 0.3 per cent w/v solution in *0.1N sodium hydroxide*, Appendix 5.12.

Bis(trimethylsilyl)acetamide : *N,O*- Bis(trimethylsilyl) acetamide; $CH_3CO{\cdot}N[Si(CH_3)_3]_2$ = 203.43

DESCRIPTION – Colourless liquid.

BOILING RANGE – 71° to 73°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.414 to 1.418, Appendix 5.14.

WT. PER ML – At 20°, 0.825 to 0.835 g, Appendix 5.19.

Bismuth Oxynitrate : Bismuth Oxide Nitrate

Contains 70.0 to 74.0 per cent of Bi.

DESCRIPTION – White, microcrystalline powder.

SOLUBILITY – Practically insoluble in *water* and in *alcohol*; freely soluble in *dilute nitric acid* and in *dilute hydrochloric acid*.

ASSAY – Weigh accurately about 1 g and dissolve in a mixture of 20 ml of *glycerin* and 20 ml of *water*. Add 0.1 g of *sulphamic acid* and titrate with *0.05 M disodium ethylenediaminetetraacetate*, using *catechol violet solution* as indicator. Each ml of *0.05 M disodium ethylenediaminetetraacetate* is equivalent to 0.01045 g of Bi.

Blue Tetrazolium : Tetrazolium Blue; 3,3'-Dianisole-bis[4,4'(3,5-diphenyl)tetrazolium chloride] $C_{40}H_{32}N_8O_2Cl_2$ = 727.65.

DESCRIPTION – Lemon-yellow crystals.

SOLUBILITY – Slightly soluble in *water*; freely soluble in *chloroform* and in *methyl alcohol*; insoluble in *acetone* and in *solvent ether*.

CLARITY AND COLOUR OF SOLUTION – A 1 per cent w/v solution in *methyl alcohol* is clear. *Extinction* of the solution in 1-cm cell at 525 nm, using *water* as the blank does not exceed 0.20, Appendix 5.15 A

SUITABILITY TEST

STANDARD PREPARATION – Dissolve in *aldehyde-free alcohol* a suitable quantity of *Hydrocortisone R.S.*, previously dried at 105° for three hours and accurately weighed, and prepare by stepwise dilution a solution containing about 10 µg per ml.

PROCEDURE – Take 10 ml, 15 ml and 20 ml of the *standard preparation* into three 50-ml stoppered conical flasks. Add 10 ml and 5 ml respectively of *aldehyde-free alcohol* to the flasks containing 10 ml and 15 ml portions of the standard preparation. To each flask and to a fourth flask containing 20 ml of *aldehyde-free alcohol*, add 2.0 ml of a 0.5 per cent w/v solution of the blue tetrazolium in *aldehyde-free alcohol*, mix, and add 2.0 ml of a solution prepared by mixing 1 volume of *tetramethylammonium hydroxide solution (10 per cent)* and 9 volumes of *aldehyde-free alcohol*. Mix, allow to stand in the dark for ninety minutes, and measure the *extinctions* in 1-cm cell at 525 nm, Appendix 5.15 A, of the three solutions containing standard preparation, using the solution in the fourth flask as the blank. Plot the extinctions on the abscissa and the amount of hydrocortisone in each flask on the ordi-

nate scale of arithmetic coordinate paper; the *extinction* of each solution is proportional to the concentration, and the *extinction* of the solution containing 200 µg of the hydrocortisone is not less than 0.5.

Blue Tetrazolium Solution

Dissolve 50 mg of *blue tetrazolium* in 10 ml of *aldehyde-free ethyl alcohol*.

Blue Tetrazolium Solution, Alkaline

Immediately before use mix one volume of a 0.2 per cent w/v solution of *blue tetrazolium* in *methyl alcohol* with three volumes of a 12 per cent w/v solution of *sodium hydroxide* in *methyl alcohol*.

Borax : Sodium Tetraborate; $Na_2B_4O_7,10H_2O.$ = 381.37

Contains not less than 99.0 per cent and not more than the equivalent of 103.0 per cent of $Na_2B_4O_7,10H_2O$.

DESCRIPTION – Transparent, colourless crystals, or a white, crystalline powder; odourless; taste, saline and alkaline. Effloresces in dry air, and, on ignition, loses all its water of crystallisation.

SOLUBILITY – Soluble in *water*, practically insoluble in *alcohol*.

ALKALINITY – A solution is alkaline to *litmus solution*.

ARSENIC – Not more than 10 parts per million, Appendix 3.2.1.

HEAVY METALS – Dissolve 1 g in 16 ml of *water* and 6 ml of *N hydrochloric acid* and add *water* to make 25 ml; the limit of heavy metals is 20 parts per million, Appendix 3.2.4.

IRON – 0.5 g complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDES – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATES – 1 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 3 g and dissolve in 75 ml of *water* and titrate with *0.5N hydrochloric acid*, using *methyl red solution* as indicator. Each ml of *0.5N hydrochloric acid* is equivalent to 0.09534 g of $Na_2B_4O_7,10H_2O$.

STORAGE – Preserve Borax in well-closed container.

Boric Acid

Of the Indian Pharmacopoeia.

Brilliant Green : CI 42040; Malachite green G; CI basic green; $C_{27}H_{34}N_2O_4S$ = 482.64

DESCRIPTION – Small, glistening, golden crystals.

SOLUBILITY – Soluble in *water* and in *alcohol*.

ABSORPTION MAXIMUM – 623 nm, Appendix 5.15 A.

Brilliant Green Solution

A 0.5 per cent w/v solution of *brilliant green* in *glacial acetic acid*.

Brilliant Yellow : Sodium 4,4′-di(4-hydroxyphenylazo)-stilbene-2,2′-disulphonate; $C_{22}H_{18}N_4Na_2O_8S_2$ = 576.51

DESCRIPTION – Reddish-brown powder.

SOLUBILITY – 0.1 g dissolves in 100 ml of *alcohol (20 per cent)* to give an orange solution.

Brilliant Black BN : CI 28440; $C_{28}H_{17}N_5Na_4O_{14}S_4$ = 867.67

DESCRIPTION – Bluish-violet or greyish-black powder.

It complies with the following test:

HOMOGENEITY – Carry out the test for separating power described under *cellulose MN 300*, applying to the plate a solution containing only the brilliant black BN; the chromatogram shows only one spot at an R_f of about 0.5.

Bromine : Br_2 = 159.80

DESCRIPTION – Reddish-brown, fuming, corrosive liquid.

SOLUBILITY – Slightly soluble in *water*; soluble in most organic solvents.

IODINE – Boil 0.2 ml with 20 ml of *water*, 0.2 ml of *N sulphuric acid* and a small piece of marble until the liquid is almost colourless. Cool, add one drop of *liquefied phenol*, allow to stand for two minutes, and then add 0.2 g of *potassium iodide* and 1 ml of *starch solution*; no blue colour is produced.

ARSENIC – Not more than 1 part per million, Appendix 3.2.1.

SULPHATE – Shake 3 ml with 30 ml of dilute *ammonia solution* and evaporate to dryness on a water-bath, the residue complies with the *limit test for sulphates*, Appendix 3.2.8.

Bromine-Sodium Acetate Solution

Dissolve 100 g of *sodium acetate* in 1000 ml of *glacial acetic acid* add, 50 ml of *bromine*, and mix.

Bromine Solution

Dissolve 9.6 ml of *bromine* and 30 g of *potassium bromide* in sufficient *water* to produce 100 ml.

Bromine Water

A freshly prepared saturated solution obtained by shaking occasionally during twenty-four hours 3 ml of *bromine* with 100 ml of *water*, and allowing to separate.

STORAGE – Store in solution over an excess of bromine.

Butyl Acetate : $CH_3.CO.O.(CH_2)_3.CH_3 = 116.16$

Contains not less than 97.0 per cent w/w of $C_6H_{12}O_2$.

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

SOLUBILITY – Very slightly soluble in *water*.

DISTILLATION RANGE – Not less than 95 per cent distils between 124° and 127°, Appendix 5.3.

WT. PER ML – Between 0.878 and 0.880 g, Appendix 5.19.

ASSAY – Carry out the method for the *determination of esters*, Appendix 3.3.2, using 1.5 g and 50 ml of *0.5N alcoholic potassium hydroxide*. Each ml of *0.5N alcoholic potassium hydroxide* is equivalent to 0.05808 g of $C_6H_{12}O_2$.

***n*-Butyl Alcohol** : Butyl Alcohol; Butan-1-ol; $CH_3.CH_2.CH_2.CH_2OH = 74.12$

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Freely soluble in *water*.

DISTILLATION RANGE – Not less than 95 per cent distils between 115° and 118°, Appendix 5.3.

WT. PER ML – At 25°, about 0.81 g, Appendix 5.19.

FLUORESCENCE – When examined under screened ultraviolet light, it shows no fluorescence.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

***s*-Butyl Alcohol** : Butan-2-ol; $CH_3.CH_2.CHOH.CH_3 = 74.12$.

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Miscible with *alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 98.5° and 100°, Appendix 5.3.

WT. PER ML – At 20°, about 0.81 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.005 per cent w/v of residue.

***t*-Butyl Alcohol** : 2-Methylpropan-2-ol; $(CH_3)_3.COH = 74.12$

DESCRIPTION – Colourless liquid or solid.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 81° and 83°, Appendix 5.3.

MELTING RANGE – 24° to 26°, Appendix 5.11.

WT. PER ML – At 26°, 0.778 to 0.782 g, Appendix 5.19.

NON-VOLATILE MATTER – Leaves not more than 0.005 per cent w/v of residue, when evaporated on a water-bath and dried to constant weight at 105°.

Butylated Hydroxytoluene : Di-*t*-butyl-*p*-cresol

Of the Indian Pharmacopoeia.

Cadmium Acetate : $(CH_3{\cdot}CO_2)_2Cd, 2H_2O = 266.53$

Contains not less than 98.0 per cent of $(CH_3{\cdot}CO_2)_2Cd, 2H_2O$.

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *water*.

ASSAY – Dissolve 1 g in 50 ml of *water*, add 25 ml of *strong ammonia solution*, and titrate with *0.1M disodium ethylenediaminetetraacetate*, using *methyl thymol blue mixture* as indicator, until the blue solution becomes colourless or grey. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02665 g of $(CH_3{\cdot}CO_2)_2Cd, 2H_2O$.

Cadmium Iodide : $CdI_2 = 366.23$

DESCRIPTION – Pearly white flakes or a crystalline powder.

SOLUBILITY – Freely soluble in *water*.

IODATE – Dissolve 0.2 g in 10 ml of *water*, and add 0.5 g of *citric acid* and 1 ml of *starch solution*; no blue colour is produced.

Cadmium Iodide Solution

A 5.0 per cent w/v solution of *cadmium iodide* in *water*.

Cadmium and Ninhydrin Solution

Dissolve 50 mg of *cadmium acetate* in a mixture of 5 ml of *water* and 1 ml of *glacial acetic acid* and dilute with *ethyl methyl ketone* to 50 ml. Immediately before use add and dissolve sufficient *ninhydrin* to produce a solution containing 0.2 per cent w/v.

Calcium Carbonate

Of the Indian Pharmacopoeia.

Calcium Chloride

Of the Indian Pharmacopoeia.

Calcium Chloride, 0.025 M

Dissolve 3.675 g of *calcium chloride* in *water* and dilute with sufficient *water* to produce 1000 ml.

Calcium Chloride, 0.05 M

Dissolve 7.35 g of *calcium chloride* in *water* and dilute with sufficient *water* to produce 1000 ml.

Calcium Chloride, Anhydrous : $CaCl_2 = 110.99$

Contains not less than 98.0 per cent of $CaCl_2$, calculated with reference to the substance dried to constant weight at 200°.

DESCRIPTION – Dry, white granules; very deliquescent.

SOLUBILITY – Freely soluble in *water*, and in *alcohol*.

FREE ALKALI – A solution of 2 g in 20 ml of *water* is not more than slightly turbid, and requires for neutralisation not more than 2.0 ml of *0.1N hydrochloric acid*, using *bromothymol blue solution* as indicator.

LOSS ON DRYING – When dried to constant weight at 200°, loses not more than 10 per cent of its weight, Appendix 5.8.

ASSAY – Dissolve about 3 g, accurately weighed, in sufficient *water* to produces 500 ml; dilute 25 ml to 100 ml with *water*, and complete the **Assay** described under Calcium Carbonate, beginning with the words "Titrate with ..... ". Each ml of *0.05 M disodium ethylenediaminetetraacetate* is equivalent to 0.005550 g of $CaCl_2$.

Calcium Chloride Solution

A 10.0 per cent w/v solution of *calcium chloride* in *water*.

Calcium Hydroxide

Of the Indian Pharmacopoeia.

Calcium Hydroxide Solution

Approximately 0.15 per cent w/v solution of *calcium hydroxide* in *water*.

Calcium Oxide : Quicklime; $CaO = 56.08$

DESCRIPTION – Dry, white lumps or powder; it readily absorbs moisture and carbon dioxide from the atmosphere. When moistened with *water*, a reaction takes place with the evolution of heat and the lumps swell and fall to powder forming calcium hydroxide.

LOSS ON IGNITION – When ignited strongly, loses not more than 10 per cent of its weight.

Calcium Solution FP

Dissolve 1.001 g of *calcium carbonate*, previously dried to constant weight at 105°, in 25 ml of *N hydrochloric acid*; boil to remove carbon dioxide, and add sufficient freshly distilled *water* to produce 1000.0 ml. Calcium Solution FP contains 400 µg of calcium per ml.

Calcium Sulphate : $CaSO_4,2H_2O = 172.17$

DESCRIPTION – White powder.

SOLUBILITY – Slightly soluble in *water*.

CHLORIDE – Boil 5 g with 50 ml of *water* and filter while hot. The filtrate, after cooling, complies with the *limit test for chlorides*, Appendix 3.2.2.

ACID-INSOLUBLE MATTER : Boil 2 g with 100 ml of *N hydrochloric acid*, filter, wash with hot *dilute hydrochloric acid* and then with *water*, dry, ignite, and weigh ; the residue weighs not more than 2 mg.

ALKALINITY : Boil 1 g with 50 ml of *water*, cool, and titrate with *0.1N hydrochloric acid*, using *bromothymol blue solution* as indicator; not more than 0.3 ml of *0.1N hydrochloric acid* is required.

CARBONATE : Boil 1 g with 10 ml of *water* and add 1 ml of *hydrochloric acid*; no carbon dioxide is evolved.

RESIDUE ON IGNITION : When ignited, leaves not less than 78.5 per cent and not more than 80.0 per cent of residue.

Calcium Sulphate Hemihydrate

Plaster of Paris of the Indian Pharmacopoeia.

Calcium Sulphate Solution

A saturated solution of calcium sulphate in *water*.

Calcon Mixture

A mixture of 1 part of *calcon* with 99 parts of freshly ignited *anhydrous sodium sulphate*.

SENSITIVITY TO CALCIUM – Dissolve 0.2 gm in 5 ml of *water*. To 1 ml of the solution add 50 ml of *water* 1 ml of *magnesium sulphate*; the solution is blue. Add 0.1 ml of a 0.15 per cent w/v solution *calcium chloride*; the solution becomes violet and on subsequent addition of 0.1 ml of 0.01 M *desodium edetate vs* turns to purple blue.

Camphor : $C_{10}H_{16}O = 152.23$

Camphor is a ketone, obtained from *Cinnamomum camphora* (Linn.) Nees and Eberm. (Fam. Lauraceae) and *Ocimum kilimandscharicum* Guerke (Fam. Labiatae) (Natural Camphor) or produced synthetically (Synthetic Camphor). It contains not less than 96.0 per cent of $C_{10}H_{16}O$.

DESCRIPTION – Colourless or white crystals, granules or crystalline masses or colourless to white, translucent tough masses; odour, penetrating and characteristic; taste, pungent, aromatic, and followed by a sensation of cold.

Readily pulverisable in the presence of a little *alcohol*, *chloroform*, or *solvent ether*.

SOLUBILITY – Slightly soluble in *water*; very soluble in *alcohol*, in *chloroform* and in *solvent ether*; freely soluble in fixed oils and in volatile oils.

MELTING RANGE – 174° to 179°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – +41° to +43°, determined in a 10 per cent w/v solution of Natural Camphor in *alcohol*, Appendix 5.12. Synthetic Camphor is the optically inactive, racemic form.

WATER – A 10 per cent w/v solution in *light petroleum (boiling range 40° to 60°)* is clear.

NON-VOLATILE MATTER – Leaves not more than 0.05 per cent of residue when volatilised at105°.

ASSAY – Weigh accurately about 0.2 g and dissolve in 25 ml of *aldehyde-free alcohol*, in a 300-ml flask. Slowly add while stirring 75 ml of *dinitrophenylhydrazine solution* and heat on a water-bath for four hours under a reflux condenser. Remove the alcohol by distillation, allow to cool, dilute to 200 ml with a 2 per cent v/v solution of *sulphuric acid* in *water*. Set aside for twenty-four hours, filter in a tared Gooch crucible, and wash the precipitate with successive quantities of 10 ml of cold *water* until the washings are neutral of *litmus paper*. Dry to constant weight at 80° and weigh. Each g of precipitate is equivalent to 0.458 g of $C_{10}H_{16}O$.

STORAGE – Preserve Camphor in a well-closed container in a cool place.

Carbon Dioxide : $CO_2 = 44.01$

Commercially available carbon dioxide.

Carbon Disulphide : $CS_2 = 76.14$

DESCRIPTION – Clear, almost colourless, flammable liquid.

DISTILLATION RANGE – Not less than 95 per cent distils between 46° and 47°, Appendix 5.3.

WT. PER ML – At 25°, about 1.263 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath, and dried to constant weight at 105°, leaves not more than 0.005 per cent w/v of residue.

Carbon Tetrachloride : $CCl_4 = 153.82$

DESCRIPTION – Clear, colourless, volatile liquid; odour, characteristic.

SOLUBILITY – Practically insoluble in *water*; miscible with *ethyl alcohol*, and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 76° and 77°, Appendix 5.3.

WT. PER ML – At 20°, 1.592 to 1.595 g, Appendix 5.19.

CHLORIDE; FREE ACID – Shake 20 ml with 20 ml of freshly boiled and cooled *water* for three minutes and allow separation to take place; the aqueous layer complies with the following tests:

Chloride – To 10 ml add one drop of *nitric acid* and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free acid – To 10 ml add a few drops of *bromocresol purple solution*; the colour produced does not indicate more acidity than that indicated by the addition of the same quantity of the indicator to 10 ml of freshly boiled and cooled *water*.

FREE CHLORINE – Shake 10 ml with 5 ml of *cadmium iodide solution* and 1 ml of *starch solution*; no blue colour is produced.

OXIDISABLE IMPURITIES – Shake 20 ml for five minutes with a cold mixture of 10 ml of *sulphuric acid* and 10 ml of *0.1N potassium dichromate*, dilute with 100 ml of *water* and add 3 g of *potassium iodide*; the liberated iodine requires for decolorisation not less than 9 ml of *0.1N sodium thiosulphate*.

NON-VOLATILE MATTER : Leaves on evaporation on a water-bath and drying to constant weight at 105°, not more than 0.002 per cent w/v of residue.

Carbon Tetrachloride I.R.

Use spectroscopic reagent grade.

Carmine

A red colouring matter obtained from cochineal.

DESCRIPTION – Bright red granular lumps or powder.

SOLUBILITY – Almost completely soluble in *strong ammonia solution*, forming a deep crimson solution.

LOSS ON DRYING – Loses not more than 20.0 per cent of its weight when dried to constant weight at 100°, Appendix 5.8.

Carmine Fibrin

Soak-washed and shredded *fibrin* for twenty-four hours in a solution prepared by dissolving 1 g of *carmine* in 1 ml of *strong ammonia solution* and diluting with 400 ml of *water*, strain; wash the fibrin with *water*, until the washings are colourless.

STORAGE – Store Carmine Fibrin under *solvent ether*.

Casein, Purified

DESCRIPTION – White or slightly yellow, odourless, granular powder.

SOLUBILITY – Insoluble in *water* and in other neutral solvents.

SOLUBILITY IN ALKALI HYDROXIDES – Dissolve 4 g in a mixture of 3 ml of *N sodium hydroxide* and 90 ml of *water*. Adjust the pH of the solution to 8.7. The resulting solution is not more than faintly opalescent.

LOSS ON DRYING – Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

FAT – Dissolve 1 g in a mixture of 10 ml of *water* and 5 ml of *alcoholic ammonia solution* and shake with two quantities each of 20 ml of *hexane*. Evaporate the hexane extracts at a low temperature, and dry at 80°; the weight of the residue is not more than 2 mg.

SULPHATED ASH – Not more than 1.5 per cent, Appendix 3.2.7.

NITROGEN CONTENT – Between 15.0 per cent and 16.0 per cent, calculated with reference to the dried material, determined by the method for *determination of nitrogen, Method C*, Appendix 3.3.5.

NOTE–Casein, according to Hammersten, which is commercially available may be used.

Casein Solution

Weigh accurately 4 g of *purified casein* and dissolve by shaking with 90 ml of *water*, adjust the pH to 7.0, and dilute with sufficient *water* to 100 ml.

Casein, Vitamin-free

Use *purified casein* that has been rendered free from the fat-soluble vitamins by continuous extraction with hot *alcohol* for forty-eight hours followed by air-drying to remove the solvent.

Casein Hydrolysate, Acid-digested

DESCRIPTION – Pale yellow, granular powder; odour, characteristic; hygroscopic.

CLARITY AND COLOUR OF SOLUTION – A 2 per cent w/v solution in *carbon dioxide-free water* is clear and does not contain suspended matter.

pH – Between 6.8 and 7.5, determined in a 2 per cent w/v solution in *carbon dioxide-free water*, Appendix 5.10.

IRON – Not more than 20 parts per million.

Dissolve the residue obtained in the test for **ASH** in 10 ml of *dilute hydrochloric acid* and 20 ml of *water*; the resulting solution complies with the *limit test for iron*, Appendix 3.2.5.

SODIUM CHLORIDE – Not more than 15.0 per cent.

Weigh accurately about 0.5 g and dissolve in 100 ml of *water*. Add one drop of *glacial acetic acid*, and titrate with *0.1N silver nitrate*, determining the end-point potentiometrically, using a silver-silver chloride electrode system. Each ml of *0.1N silver nitrate* is equivalent to 0.005844 g of NaCl.

TOTAL NITROGEN – Not less than 8.0 per cent.

Weigh accurately about 0.2 g and carry out the *determination of nitrogen, Method A*, Appendix 3.3.5.

ASH – Not more than 25 per cent, determined by igniting 2.0 g, accurately weighed, at a temperature not exceeding 600°.

LOSS ON DRYING – Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°, Appendix 5.8.

Catechol : Pyrocatechol; *o*-Dihydroxybenzene; $C_6H_4(OH)_2 = 110.11$

DESCRIPTION – White, crystalline powder, becoming tinted on exposure to light or air.

SOLUBILITY – Freely soluble in *water*, in *alcohol*, in *benzene*, in *chloroform*, in *solvent ether* and in *pyridine*. Its aqueous solution soon turns brown.

MELTING RANGE – 101° to 105°, Appendix 5.11.

SULPHATED ASH – Not more than 0.2 per cent, Appendix 3.2.7.

Catechol Solution

A freshly prepared 10.0 per cent w/v solution of *catechol* in *water*.

Cellulose MN 300

A fine, white, homogeneous powder of an average particle size of less than 30 µm. It complies with the following requirement:

SEPARATING POWER – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using a suspension of 25 g in 90 ml of *water* as the coating medium and a mixture of 50 volumes of *n-propyl alcohol*, 10 volumes of *ethyl acetate* and 40 volumes of *water* as the mobile phase. Apply to the plate 10 µl of a solution in equal volumes of *methyl alcohol* and *water* containing 0.025 per cent w/v of each of *brilliant black BN*, *amaranth S*, *fast yellow AB* and *tropaeolin O*. Allow the mobile phase to ascend 10 cm. The chromatogram shows four clearly separated spots, one black, one red and two yellow in order of increasing R_f value.

Cellulose, Microcrystalline

Of the Indian Pharmacopoeia

Ceric Ammonium Sulphate : Ammonium Ceric Sulphate; $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O = 632.53$

Contains not less than 95.0 per cent of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

DESCRIPTION – Yellow or orange-yellow crystals, or a crystalline powder.

SOLUBILITY – Slightly soluble in *water*; practically insoluble in *alcohol*.

ASSAY – Weigh accurately about 1 g and dissolve in *dilute sulphuric acid*, add 3 g of *potassium iodide*, and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.06326 g of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

Cerous Nitrate : $Ce(NO_3)_3,6H_2O = 434.15$

Contains not less than 98.0 per cent of $Ce(NO_3)_3,6H_2O$.

DESCRIPTION – Colourless or pale yellow, moist crystalline powder.

SOLUBILITY – Soluble in *water*.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – To 1.5 g, accurately weighed, add 5 ml of *sulphuric acid and heat*, until copious white fumes are evolved, cool, add 30 ml of *sulphuric acid*, cool, add 2 drops of a 1 per cent solution of *osmium tetraoxide*, and titrate with *0.1N sodium arsenite*, using *ferroin sulphate solution* as indicator. Each ml of *0.1N sodium arsenite* is equivalent to 0.04342 g of $Ce(NO_3)_3,6H_2O$.

Cerous Nitrate Solution

Dissolve 0.22 g of *cerous nitrate* in 50 ml of *water*, add 0.1 ml of *nitric acid*, 50 mg of *hydroxylamine hydrochloride* and sufficient *water* to produce 1000 ml.

Cetrimide

Of the Indian Pharmacopoeia.

Charcoal, Decolorising

DESCRIPTION – Fine, black powder.

DECOLORISING POWER – Add 0.1 g to 50 ml of a 0.006 per cent w/v solution of *bromophenol blue* in *alcohol (20 per cent)* contained in a 250-ml flask, and mix by rotating the vessel, allow to stand for five minutes, and filter; the colour of the filtrate is not deeper than that of a solution prepared by diluting 1 ml of the solution of bromophenol blue to 50 ml with *alcohol (20 per cent)*.

ACID-SOLUBLE MATTER—Heat 1 g with 10 ml of *dilute sulphuric acid* and 20 ml of *water* for five minutes on a water-bath. Filter, evaporate the filtrate to dryness, ignite and weigh; the residue weighs not more than 25 mg.

SULPHATED ASH – Not more than 5.0 per cent, Appendix 3.2.7.

Charcoal, Activated

Use *decolorising charcoal*.

Chloral Hydrate

Of the Indian Pharmacopoeia.

Chloralose : $C_8H_{11}Cl_3O_6 = 309.54$.

DESCRIPTION – A colourless, crystalline powder.

MELTING POINT – About 187°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – +19°, determined in a 5.0 per cent w/v solution in *alcohol (75 per cent)*, Appendix 5.12.

Chloramine : Chloramine T; Sodium Salt of *N*-Chlorotoluene-*p*-sulphonamide; $C_7H_7ClNNaO_2S,3H_2O = 281.72$
Contains not less than 98.0 per cent of $C_7H_7ClNNaO_2S,3H_2O$.

DESCRIPTION – White crystals or a white crystalline powder; odour, that of chlorine; efflorescent; decomposed slowly by *alcohol*.

SOLUBILITY – Freely soluble in *water*; soluble in *alcohol*; highly soluble in boiling *water*; insoluble in *solvent ether* and in *chloroform*.

ALKALINITY – A solution is slightly alkaline to *phenolphthalein solution*.

ORTHO COMPOUND – Mix about 2 g with 10 ml of *water*, add about 1 g of *sodium pyrosulphite* and boil. Cool to 0°, filter rapidly, and wash with three quantities, each of 5 ml, of ice-cold *water*. Dry the washed precipitate over *phosphorus pentoxide* at a pressure not exceeding 5 mm of mercury.

MELTING POINT – Not lower than 134°, Appendix 5.11.

SODIUM CHLORIDE – 1.0 g, treated with 15 ml of *ethyl alcohol* without the aid of heat, leaves not more than 15 mg of insoluble residue.

ASSAY – Weigh accurately about 0.4 g and dissolve in 50 ml of *water* in a glass-stoppered vessel. Add 10 ml of *potassium iodide solution* and 5 ml of *dilute sulphuric acid*. Allow the mixture to stand for about ten minutes and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.01408 g of $C_7H_7ClNNaO_2S,3H_2O$.

Chloramine Solution

A 2.0 per cent w/v solution of *chloramine* in *water*.

Chlorimide Solution

A 0.4 per cent w/v solution of *2,6-dibromoquinone chlorimide* in *ethyl alcohol*.

Chlorinated Lime : Bleaching Powder

Contains not less than 30.0 per cent w/w of available chlorine.

DESCRIPTION – Dry, dull-white powder; odour, characteristic.

On exposure to air it becomes moist and gradually decomposes.

SOLUBILITY – Slightly soluble in *water* and in *alcohol*.

STABILITY – Loses not more than 3.0 per cent of its available chlorine by weight when heated to 100° for two hours (The available chlorine is determined by the ASSAY described below).

ASSAY – Weigh accurately about 4 g, triturate in a mortar with successive small quantities of *water*, and transfer to a 1000-ml flask. Add sufficient *water* to produce 1000 ml and shake thoroughly. To 100 ml of this suspension add 3 g of *potassium iodide* dissolved in 100 ml of *water*, acidify with 5 ml of *acetic acid*, and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003545 g of available chlorine.

STORAGE – Preserve in a well-closed container.

Chlorinated Lime Solution

Mix 100 g of *chlorinated lime* with 1000 ml of *water*; transfer the mixture to a stoppered bottle; set aside for three hours, shaking occasionally; filter through calico.

Chlorinated Lime Solution must be recently prepared.

Chlorinated Soda Solution

Dissolve 150 g of *sodium carbonate* in 250 ml of *water*; thoroughly triturate 100 g of *chlorinated lime* with 750 ml of *water*; mix the two liquids, shake occasionally during three to four hours, and filter.

Chlorinated Soda Solution must be recently prepared.

Chlorine : $Cl_2 = 70.91$

The gas obtained by reacting *hydrochloric acid*, or a chloride and *sulphuric acid*, with an oxidising agent such as *manganese dioxide*.

Chlorine Solution

A saturated solution of *chlorine* in *water*.

Chlorine Solution, Dilute

Dilute 33 ml of *chlorine solution* to 100 ml with *water*.

Dilute Chlorine Solution must be freshly prepared.

4-Chloroacetanilide : $CH_3.CO.NH.C_6H_4.Cl = 169.61$

Contains not less than 98.0 per cent of C_8H_8ClNO, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – Colourless, rhombic crystals or plates.

SOLUBILITY – Slightly soluble in *water* and in *alcohol*.

MELTING RANGE – 176° to 178°, Appendix 5.11.

LOSS ON DRYING – Loses not more than 2.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1.5 g and dissolve in 20 ml of *alcohol*, add 3.5 g of *Raney nickel catalyst*, and connect to a reflux condenser. Add 75 ml of a 10 per cent w/v solution of *sodium hydroxide* down the condenser, cool the flask in water until the initial reaction has subsided, and heat over a very small flame for four hours. Cool, decant the solution, wash the residue with hot *water*, decant the washings, and dilute the combined solution and washings to 200 ml with *water*. Acidify 50 ml of this solution to *congo red paper* with a mixture of equal volumes of *nitric acid* and *water*, add 50 ml of *0.1N silver nitrate*, shake well, and titrate the excess of *silver nitrate* with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.01695 g of C_8H_8ClNO.

4-Chloroaniline : *p*-Chloroaniline; $Cl.C_6H_4.NH_2 = 127.57$

DESCRIPTION – White or faintly coloured crystals.

MELTING RANGE – 70° to 72°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7

1-Chloro-2,4-Dinitrobenzene : $Cl.C_6H_3(NO_2)_2 = 202.56$

DESCRIPTION – Pale-yellow crystals or crystalline powder.

CLARITY OF SOLUTION – 5 g dissolves in 100 ml of *alcohol* to yield a clear yellow solution.

MELTING RANGE – 50° to 52°, Appendix 5.11.

SULPHATED ASH – Not more than 0.02 per cent, Appendix 3.2.7.

Chloroform

Of the Indian Pharmacopoeia.

Chloroform, Alcohol-free

Wash repeatedly *chloroform* with *water* and dry with *anhydrous sodium sulphate* and distil.

WT. PER ML – At 20°, not less than 1.489 g, Appendix 5.19.

Alcohol-free Chloroform must be freshly prepared.

Chloroform Water

Chloroform	...	...	2.5 ml
Purified water	...	sufficient to produce	1000 ml

Dissolve the *chloroform* in the *purified water* by shaking..

4-Chlorophenol : *p*-Chlorophenol; $Cl.C_6H_4.OH = 128.56$

Contains not less than 98.0 per cent of C_6H_5ClO.

DESCRIPTION – Colourless or slightly coloured crystals.

SOLUBILITY : Soluble in *alcohol* and solutions of the alkali hydroxides.

MELTING RANGE – 41° to 43°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 2 g and dissolve in *water* and minimum amount of *sodium hydroxide solution*, and dilute to 500 ml with *water*. To 10 ml of this solution add 25 ml of *0.1N bromine*, 65 ml of *water*, and 5 ml of *hydrochloric acid*, shake in a stoppered bottle for a few minutes, and allow to stand for thirty minutes. Add 2 g of *potassium iodide* and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N bromine* is equivalent to 0.003214 g of C_6H_5ClO.

Chlorosulphonic Acid : Sulphuric Chlorohydrin; $ClSO_3H = 116.53$

Contains not less than 98.0 per cent of $ClSO_3H$, as determined by both parts of the **ASSAY** described below.

DESCRIPTION – Clear, colourless, fuming liquid.

WT. PER ML – 1.75 to 1.77 g, Appendix 5.19.

BOILING RANGE – 151° to 152°, Appendix 5.3.

IRON – 0.5 ml complies with the *limit test for iron*, Appendix 3.2.5.

ASSAY – (a) Weigh 0.8 ml into a stoppered flask containing 100 ml of *water* and titrate with *N sodium hydroxide* using *bromothymol blue solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.03884 g of $ClSO_3H$.

(b) Dilute the titrated solution from (a) to 250 ml with *water*. To 50 ml of this solution add 10 ml of a 50 per cent v/v solution of *nitric acid* and 50 ml of *0.1N silver nitrate*. Titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.01165 g of $ClSO_3H$.

Cholesterol : $C_{27}H_{46}O = 386.64$

DESCRIPTION – White or faintly yellow, almost odourless, pearly leaflets or granules.

SOLUBILITY – Practically insoluble in *water*; sparingly soluble in *ethyl alcohol*; soluble in *acetone*, in hot *alcohol*, in *chloroform*, in *dioxan*, in *solvent ether*; in *ethyl acetate* and in vegetable oils.

MELTING RANGE – 147° to 150°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – Not less than −34° and not more than −38°, Appendix 5.12, determined in 2.0 per cent solution in *dioxan*.

LOSS ON DRYING – Loses not more than 0.3 per cent of its weight when dried "in vacuo at 60°" for four hours, Appendix 5.8.

SULPHATED ASH – Not more than 0.1 per cent; Appendix 3.2.7.

ACIDITY – Dissolve 1 g in 10 ml of *solvent ether* in a small flask, add 10 ml of *0.1N sodium hydroxide*, and shake for about one minute. Heat gently to expel the solvent ether and then boil for five minutes. Cool, dilute with 10 ml of *water* and titrate with *0.1N sulphuric acid*, using 2 drops of *phenolphthalein solution* as indicator. Perform a blank determination with the same quantities of the same reagents and in the same manner omitting cholesterol. The difference between the number of ml of *0.1N sulphuric acid* consumed in the blank and the number of ml consumed in the test with the cholesterol is not more than 0.3 ml.

SOLUBILITY IN ALCOHOL—Dissolve 500 mg in 50 ml of warm *alcohol* in a stoppered flask or cylinder and allow to stand at room temperature for two hours; no deposit or turbidity is formed.

Choline Chloride : $HOCH_2.CH_2.N(CH_3)_3Cl = 139.62$

Contains not less than 99.5 per cent of $C_5H_{14}ONCl$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White crystals or crystalline powder; hygroscopic.

SOLUBILITY – Very soluble in *water*.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.1 g previously dried at 105° for two hours, dissolve in 40 ml of *water* and add 10 ml of freshly prepared *ammonium reineckate solution*

allowing it to run down the side of the beaker to form a layer under the solution of choline chloride without producing turbulence. Mix the two layers by gentle rotation and allow to stand in the cold with occasional gentle swirling. Filter through a sintered glass filter; wash the beaker and the precipitate with three 10-ml portions of a solution containing 2 ml of *ammonium reineckate solution* in 1000 ml of *water*, cooled to 5°. Dry the precipitate at 105° for one hour and weigh. The weight multiplied by 0.3304 gives the equivalent weight of $C_5H_{14}ONCl$.

STORAGE – Store Choline Chloride in tightly-closed containers.

Chromatographic Siliceous Earth

A form of SiO_2, consisting of frustules and fragments of diatoms, purified by boiling with *dilute hydrochloric acid*, washing, and calcining.

Chromic Acid Mixture

See Cleaning of Glassware, Appendix 6.1.

Chromic Acid Solution

Dissolve 84 g of *chromium trioxide* in 700 ml of *water* and add slowly, with stirring, 400 ml of *sulphuric acid*.

Chromic Sulphuric Acid

A saturated solution of *chromium trioxide* in *sulphuric acid*.

Chromium Trioxide : $CrO_3 = 99.99$

Contains not less than 95.0 per cent of CrO_3.

DESCRIPTION – Dark red, acicular crystals, or dark brown masses; odourless; deliquescent and corrosive.

SOLUBILITY – Very soluble in *water*, and in *solvent ether*.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

WATER-SOLUBLE ASH – Heat 1 g to redness, extract the residue with hot *water*, filter, evaporate the filtrate to dryness, and dry to constant weight at 105°; the residue weighs not more than 20 mg.

ASSAY – Accurately weigh about 1 g, and dissolve in sufficient *water* to produce 100 ml; to 10 ml of the solution add 10 ml of *dilute sulphuric acid* and 3 g of *potassium iodide*; allow to stand for fifteen minutes, and dilute with *water*; titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003333 g of CrO_3.

STORAGE – Chromium Trioxide should be stored in a well-closed, glass-stoppered container.

Chromotropic Acid : $C_{10}H_8O_8S_2,2H_2O = 356.32$

DESCRIPTION – White to brownish powder. It is usually available as its sodium salt, $C_{10}H_6O_8S_2Na_2$, which is yellow to light brown in colour.

SOLUBILITY – Soluble in *water*; sodium salt is freely soluble in *water*.

SENSITIVITY – Dilute exactly 0.5 ml of *formaldehyde solution* with *water* to make 1000 ml. Dissolve 5 mg of *chromotropic acid*, or its sodium salt, in 10 ml of a mixture of 9 ml of *sulphuric acid* and 4 ml of *water*. Add 5 ml of this solution to 0.2 ml of the formaldehyde solution, and heat for 10 minutes at 60°; a violet colour is produced.

Chromotropic Acid, Sodium Salt : Disodium 4,5-dihydroxynaphthalene -2.7-disulphonate; $C_{10}H_6Na_2O_8S_2 = 364.26$

DESCRIPTION; SOLUBILITY; SENSITIVITY – Complies with the requirements described under Chromotropic Acid.

Chromotropic Acid Solution

Dissolve 50 mg of *chromotropic acid* or its sodium salt in 100 ml of 75 per cent v/v of *N sulphuric acid*.

Cinchonidine : $C_{19}H_{22}N_2O = 294.38$

DESCRIPTION – White crystalline powder.

SOLUBILITY – Soluble in *alcohol*.

MELTING RANGE – 205° to 209°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – At 20°, −105° to −110°, determined in a 1.0 per cent solution in *alcohol*, Appendix 5.12.

Cinchonine : $C_{19}H_{22}N_2O = 294.38$

Contains not less than 99.0 per cent of $C_{19}H_{22}N_2O$.

DESCRIPTION – White crystalline powder.

SOLUBILITY – Soluble in *alcohol*.

MELTING RANGE – 261° to 265°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.5 g and dissolve in 50 ml of *glacial acetic acid*, warming and cooling, if necessary, and titrate with *0.1N perchloric acid* using *oracet blue B solution* as indicator. Each ml of *0.1N perchloric acid* is equivalent to 0.01472 g of $C_{19}H_{22}N_2O$.

Cineole : Eucalyptol; 1,8-Epoxy-*p*-menthane; $C_{10}H_{18}O = 154.24$

DESCRIPTION – A colourless liquid with camphoraceous odour.

SOLUBILITY – Practically insoluble in *water*; miscible with *alcohol*, with *chloroform* and with *solvent ether*.

SPECIFIC GRAVITY – Between 0.922 and 0.927 at 20°, Appendix 5.19.

REFRACTIVE INDEX – Between 1.456 and 1.459, determined at 20°, Appendix 5.14.

CONGEALING RANGE – Between 0° and 1°, Appendix 5.5.

DISTILLATION RANGE – Between 174° and 177°, Appendix 5.3.

PHENOL – Shake 1 ml with 20 ml of *water*. Allow to separate and add to 10 ml of the aqueous layer 0.1 ml of *ferric chloride test solution*. No violet colour develops.

TURPENTINE OIL – Dissolve 1 ml in 5 ml of *alcohol (90 per cent)*. Add dropwise freshly prepared *bromine water*. Not more than 0.5 ml is required to give a yellow colour lasting thirty minutes.

RESIDUE ON EVAPORATION – Not more than 0.05 per cent w/v, determined by adding 25 ml of *water* to 10.0 ml and evaporating on a water-bath and drying to constant weight at 105°.

Citric Acid

Of the Indian Pharmacopoeia.

Citric Acid, 0.5 M

Dissolve 105.07 g of *citric acid* in sufficient *water* to produce 1000 ml.

Citric Acid, Anhydrous

Of the Indian Pharmacopoeia.

Citric Acid, Iron-free

Citric acid which complies with the following additional test:

Dissolve 0.5 g in 40 ml of *water*, add 2 drops of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, and dilute to 50 ml with *water*; no pink colour is produced.

Citric-Molybdic Acid Solution

Mix 54 g of *molybdenum trioxide* with 200 ml of *water*, add 11 g of *sodium hydroxide* and heat with stirring until almost complete solution has been obtained. Dissolve 60 g of *citric acid* in 250 ml of *water* and add 140 ml of *hydrochloric acid*. Add the first solution to the second with constant stirring, cool, filter, if necessary, dilute to 1000 ml with *water*, and add dropwise, sufficient of a 1 per cent w/v solution of *potassium bromate* to discharge the green colour.

Citric-Molybdic Acid Solution should be kept in a well-closed container, protected from light.

Cobalt Acetate : $(CH_3.CO_2)_2Co,4H_2O = 249.08$

DESCRIPTION – Deep red crystals; odour, faintly acetous.

SOLUBILITY – Soluble in *water* and in *alcohol*.

INSOLUBLE MATTER – 5 g dissolved in 100 ml of *water* containing 2 ml of *glacial acetic acid*, shows not more than 1 mg of insoluble matter.

Cobalt Chloride : $CoCl_2,6H_2O = 237.93$

Contains not less than 97.5 per cent $CoCl_2,6H_2O$.

DESCRIPTION – Deep red crystals.

CLARITY OF SOLUTION – 5 g dissolves in 50 ml of *water* to yield a clear pink solution.

IRON – Dissolve 3 g in 30 ml of *water*, add 0.3 g of *zinc oxide* and boil for one minute. Filter with suction, wash the residue with *water*, dissolve in 4.5 ml of *dilute hydrochloric acid* and 30 ml of *water*, add 0.3 g of *zinc oxide*, and again boil for one minute. Filter with suction, wash the residue with *water*, dissolve in 6 ml of *dilute hydrochloric acid* and add sufficient *water* to produce 60 ml. 4 ml of this solution complies with the *limit test for iron*, Appendix 3.2.5, using 6 drops of *thioglycollic acid*.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 1 g and dissolve in 300 ml of *water*, add 2 g of *hydroxylamine hydrochloride* and 25 ml of *strong ammonia solution*, and heat to above 80°. Titrate the solution with *0.1M disodium ethylenediaminetetraacetate*, using *methyl thymol blue mixture* as indicator, until the colour changes from blue to purple. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02379 g of $CoCl_2,6H_2O$.

Cobalt Nitrate : Cobalt(II) Nitrate Hexahydrate; Cobaltous Nitrate; $Co(NO_3)_2,6H_2O = 291.00$

Contains not less than 97.5 per cent of $Co(NO_3)_2,6H_2O$.

DESCRIPTION – Red crystals, deliquescent.

SOLUBILITY – Soluble in *water*.

CHLORIDE – 3.0 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 1 g complies with the *limit test for sulphates*, Appendix 3.2.8.

IRON – Complies with the test described under Cobalt Chloride.

ASSAY – Carry out the **ASSAY** described under Cobalt Chloride. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02910 g of $Co(NO_3)_2,6H_2O$.

Cobalt Thiocyanate Reagent

Dissolve 20 g of *ammonium thiocyanate* and 5 g of *cobalt nitrate* in 100 ml of *water*. Add sufficient *sodium chloride* to saturate the solution.

Cobalt Thiocyanate Solution

Dissolve 6.8 g of *cobalt chloride* and 4.3 g of *ammonium thiocyanate* in sufficient *water* to produce 100 ml.

Copper : Cu

The pure metal known commercially under the term 'electrolytic'.

DESCRIPTION – Usually in the form of turnings or borings.

Copper Acetate : $Cu(C_2H_3O_2)_2,H_2O = 199.65$

Contains not less than 98.0 per cent of $C_4H_6O_4Cu,H_2O$.

DESCRIPTION – Blue-green crystals or powder, having a faint odour of acetic acid.

SOLUBILITY – Soluble in *water*, yielding a clear solution.

CHLORIDE – 3 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 3 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 0.8 g and dissolve in 50 ml of *water*, add 2 ml of *acetic acid* and 3 g of *potassium iodide*, and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator, until only a faint blue colour remains; add 2 g of *potassium thiocyanate* and continue the titration until the blue colour disappears. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.01997 g of $C_4H_6O_4Cu,H_2O$.

Copper Nitrate : $Cu(NO_3)_2,3H_2O = 241.56$

Contains not less than 95.0 per cent of $Cu(NO_3)_2,3H_2O$.

DESCRIPTION – Blue crystals; odour, that of nitric acid; hygroscopic.

SOLUBILITY – Freely soluble in *water* and in *alcohol*.

BARIUM – 1 g, dissolved in 50 ml of *water*, shows no turbidity after the addition of 2 ml of *dilute sulphuric acid* and standing for two hours.

CHLORIDE – 3 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 1 g, dissolved in 50 ml of *water* and 1 ml of *hydrochloric acid*, shows no turbidity after the additon of 2 ml of *barium chloride solution* and standing for two hours.

ASSAY – Weigh accurately about 1 g and dissolve in 50 ml of *water*, add 2 g of *sodium acetate*, 2 ml of *acetic acid* and 3 g of *potassium iodide*, and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator, until only a faint blue colour remains; add 2 g of *potassium thiocyanate* and continue the titration until the blue colour disappears. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.02416 g of $Cu(NO_3)_2,3H_2O$.

Copper Nitrate Solution, Ammoniacal

Dissolve 1 g of *copper nitrate* in *water*, add 10 ml of *strong ammonia solution* and sufficient *water* to produce 100 ml.

Copper Reagent

Dissolve, with the aid of heat, 150 g of *sodium citrate*, 130 g of *anhydrous sodium carbonate*, and 10 g of *sodium bicarbonate* in about 650 ml of *water*, cool, add a solution containing 16 g of *copper sulphate* in 150 ml of *water*, dilute to 1000 ml with *water* and filter.

Copper Solution, Alkaline

Dissolve 24 g of *anhydrous sodium carbonate* and 12 g of *sodium potassium tartrate* in 250 ml of *water*. Add, with stirring, 4 g of *copper sulphate* dissolved in about 50 ml of *water*, followed by 16 g of *anhydrous sodium carbonate*. Dissolve 180 g of *anhydrous sodium sulphate* in about 500 ml of *water*, boil to expel air, cool and mix with the other solution. Dilute to 1000 ml with *water*, keep aside for a few days and filter, if necessary.

STORAGE – Store in a warm place.

Copper Sulphate : $CuSO_4,5H_2O = 249.68$

Contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of $CuSO_4,5H_2O$.

DESCRIPTION – Blue triclinic prisms or a blue, crystalline powder.

SOLUBILITY – Soluble in *water*, very soluble in boiling *water*, almost insoluble in *alcohol*; very slowly soluble in *glycerin*.

ACIDITY AND CLARITY OF SOLUTION – 1 g, dissolved in 20 ml of *water*, forms a clear blue solution, which becomes green on the addition of 0.1 ml of *methyl orange solution*.

IRON – To 5 g, add 25 ml of *water*, and 2 ml of *nitric acid*, boil and cool. Add excess of *strong ammonia solution*, filter, and wash the residue with *dilute ammonia solution* mixed with four times its volume of *water*. Dissolve the residue, if any, on the filter with 2 ml of *hydrochloric acid*, diluted with 10 ml of *water*; to be acid solutions add *dilute ammonia solution* till the precipitation is complete; filter and wash; the residue after ignition weighs not more than 7 mg.

LEAD AND ZINC – Dissolve 1 g in 10 ml of *water*, add 1 g of *citric acid* and 10 ml of *dilute ammonia solution*. Add *potassium cyanide solution* so that the blue colour is completely discharged, then add 1 drop of *sodium sulphide solution*; no opalescence or not more than a slight darkening is produced.

ASSAY – Weigh accurately about 1 g and dissolve in 50 ml of *water*, add 3 g of *potassium iodide*, 5 ml of *acetic acid*, and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Continue titration till a faint blue colour remains, add 2 g of *potassium thiocyanate*, stir well, and continue the titration until the blue colour disappears. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.02497 g of $CuSO_4,5H_2O$.

Copper Sulphate, 0.02 M

Dissolve 4.9936 g of *copper sulphate* in sufficient *water* to produce 1000 ml.

Copper Sulphate Solution

A 10.0 per cent w/v solution of *copper sulphate* in *water*.

Copper Sulphate Solution, Dilute

A 0.00393 per cent w/v solution of *copper sulphate* in *water*.

Dilute Copper Sulphate Solution contains 0.01 mg of Cu in 1 ml.

Copper Sulphate with Pyridine Solution

Dissolve 4 g of *copper sulphate* in 90 ml of *water* and add 30 ml of *pyridine*.

Copper Sulphate with Pyridine Solution should be freshly prepared.

Cresol

Of the Indian Pharmacopoeia.

***m*-Cresol** : 3-Methylphenol; C_7H_8O = 108.13

DESCRIPTION – Colourless or yellowish liquid; odour, phenolic.

SOLUBILITY – Sparingly soluble in *water*, and in solutions of fixed alkali hydroxides; miscible with *alcohol*, *chloroform* and *solvent ether*.

BOILING POINT – About 202°, Appendix 5.3.

***o*-Cresol** : 2-Methylphenol; C_7H_8O = 108.13

Pure, dry redistilled *o*-cresol with a congealing temperature not below 30°, Appendix 5.5.

Cupric Citrate Solution, Alkaline

Dissolve, with the aid of heat, 173 g of *sodium citrate* and 117 g of *sodium carbonate monohydrate* in about 700 ml of *water*, and filter through paper, if necessary, to obtain a clear solution. In a separate container dissolve 17.3 g of *copper sulphate* in about 100 ml of *water*, and slowly add this solution, with constant stirring, to the first solution. Cool the mixture, add *water* to make 1000 ml.

Cuprous Chloride : CuCl = 98.99

Dissolve 50 g of *copper sulphate* in 100 ml of *water*, add 200 ml of *hydrochloric acid* and 25 g of *copper*. Boil the mixture gently until the dark colour disappears and the liquid becomes clear. Pour into 2000 ml of *water* containing *sulphur dioxide*, allow the white precipitate to settle and wash several times by decantation with *water* containing *sulphur dioxide*. Filter through a sintered glass filter, wash with *ethyl alcohol* and then with *solvent ether*, taking care to draw only the minimum quantity of air through the solid. Dry the residue at 100° with frequent stirring until the ether is completely removed, transfer the product to ampoules and seal immediately.

Cuprous Chloride Solution

Dissolve 1.25 g of *cuprous chloride*, removed from a sealed container immediately before use, in 100 ml of *ammonium chloride solution* containing 1 g of *sodium metabisulphite*; filter if necessary.

Cuprous Chloride Solution should be freshly prepared.

Curcumin : 1,7-Bis(4-hydroxy-3-methoxyphenyl) Hepta-1,6-dien-3,5-dione; $C_{21}H_{20}O_6$ = 368.37

DESCRIPTION – Orange-brown crystalline powder.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol*.

MELTING RANGE – 180° to 186°, Appendix 5.11.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

ASSAY – Dissolve 3 mg in sufficient *alcohol* to produce 100 ml, dilute 10 ml to 100 ml with *alcohol*, and measure the *extinction* of a 1-cm layer at the maximum at about 430 nm, Appendix 5.15A. Calculate the content of $C_{21}H_{20}O_6$ taking 1600 as the value of E(1 per cent, 1-cm) at the maximum at about 430 nm.

Cyanogen Bromide : BrCN = 105.93

DESCRIPTION – Colourless crystals. Volatilises at room temperature. Its vapours are highly irritating and very toxic.

SOLUBILITY – Freely soluble in *water*, and in *alcohol*.

MELTING POINT – About 52°, Appendix 5.11.

STORAGE – Store in tightly-closed containers in a cool place.

Cyanogen Bromide Solution

Add, drop by drop, 10.0 per cent w/v solution of *potassium cyanide* in *water* to ice-cooled *bromine solution* until the colour disappears.

Cyangen Bromide Solution must be freshly prepared.

Cyclohexane : $C_6H_{12} = 84.16$

DESCRIPTION – Clear colourless liquid.

BOILING RANGE – 81° to 82°, Appendix 5.3.

CONGEALING RANGE – 4.5° to 6.5°, Appendix 5.5. .

ULTRA-VIOLET ABSORPTION – Almost completely transparent to radiation of wavelength greater than 250 nm, and exhibits no trace of discontinuous absorption, Appendix 5.15A.

WT. PER ML : At 20°, 0.776 g to 0.780 g, Appendix 5.19.

Cyclohexane UV

Use spectroscopic reagent grade.

Cysteine Hydrochloride : (–)α-Amino-β-mercaptopropionic Acid Hydrochloride; $[SH{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H]HCl = 157.66$

DESCRIPTION – White crystals.

MELTING RANGE – 175° to 178°, with decomposition, Appendix 5.11.

Cysteine Hydrochloride Solution

Dissolve 0.5 g of *cysteine hydrochloride*, accurately weighed, in 10 ml of *water*; adjust the pH to 7.0.

Cysteine Hydrochloride Solution should be freshly prepared.

L- Cystine : $HOOC(NH_2)CH{\cdot}CH_2{\cdot}S{\cdot}S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH = 240.30$

DESCRIPTION – A white, crystalline powder.

SOLUBILITY – Very slightly soluble in *water*; soluble in dilute mineral acids and in solutions of alkali hydroxides; insoluble in *alcohol* and in other organic solvents.

SPECIFIC OPTICAL ROTATION – Between −215° and −225°, determined at 20°in a 2.0 per cent w/v solution of the material, dried over *silica gel* for four hours, in *dilute hydrochloric acid*, Appendix 5.12.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 0.2 per cent, determined by drying over *silica gel* for four hours, Appendix 5.8.

Delafield's Hematoxylin Solution

Prepare 400 ml of a saturated solution of *aluminium ammonium sulphate* (solution A). Dissolve 4 g of *hematoxylin* in 25 ml of *alcohol*, mix it with solution A and allow it to stand for 4 days in a flask closed with a plug of purified cotton, and exposed to light and air (Solution B). Then filter solution B, and add to it solution C, consisting of a mixture of 100 ml of *glycerin* and 100 ml of *methyl alcohol*. Mix; and allow the mixture to stand in a warm place, exposed to light for 6 weeks until it becomes dark coloured.

STORAGE – Store in tightly-stoppered bottles.

Devarda's Alloy

Copper, 50 parts; aluminium, 45 parts; zinc, 5 parts.

DESCRIPTION – Grey powder.

SOLUBILITY – Insoluble in *water*; partly soluble in *hydrochloric acid* and in *sodium hydroxide solution*; soluble in *nitric acid*.

NITROGEN – To 7.0 g in a 100-ml long-necked flask, add, in small portions 40 ml of *hydrochloric acid*, shake frequently until hydrogen ceases to be evolved, and cool. Add 100 ml of a 30 per cent w/v solution of *sodium hydroxide* and distil 75 ml, collecting the distillate in a flask containing 10 ml of *0.02 N hydrochloric acid*. Titrate the excess of acid with *0.02 N sodium hydroxide*, using *methyl red solution* as indicator. Repeat the operation using 2 g of the alloy; the difference between the titrations is not greater than 1.8 ml.

Dextrose (Anhydrous)

Of the Indian Pharmacopoeia.

Diazobenzenesulphonic Acid Solution

Dissolve 0.9 g of *sulphanilic acid* in a mixture of 30 ml of *2N hydrochloric acid* and 70 ml of *water*. To 3 ml of the solution add 3 ml of a 5.0 per cent w/v solution of *sodium nitrite* in *water*, cool in ice-water for five minutes, add a further 12 ml of the solution of *sodium nitrite* and again cool in ice, dilute the solution with ice-cold *water* to 100 ml and allow to stand in ice for fifteen minutes.

Diazobenzenesulphonic Acid Solution must be freshly prepared and should not be used until at least fifteen minutes after its preparation.

2,6-Dibromoquinone Chlorimide : 2,6-Dibromo-*N*-chloro-*p*-benzoquinone Imine; $O{:}C_6H_2Br_2{:}NCl = 299.35$

DESCRIPTION – Yellow, crystalline powder.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol* and in dilute alkali hydroxide solution.

MELTING RANGE –Between 82° and 84°, Appendix 5.11.

SOLUBILITY IN ALCOHOL – A solution of 0.1 g in 10 ml of *alcohol* is not more than faintly turbid.

RESIDUE ON IGNITION – Ignite 500 mg with 0.5 ml of *sulphuric acid*; the residue weighs not more than 1 mg.

SENSITIVITY – To 10 ml of a 0.0001 per cent w/v solution of *phenol*, add 0.3 ml of a borax buffer (prepared by dissolving 2.84 g of *borax* in 90 ml of warm *water*, adding 8.2 ml of *N sodium hydroxide* and diluting with *water* to 100 ml) and 0.1 ml of a 0.05 per cent w/v solution of the *2,6-dibromoquinone chlorimide* in *alcohol*; a distinct blue colour develops within ten minutes.

Dibutyl Phthalate

Of the Indian Pharmacopoeia.

2,7-Dichlorofluorescein : $C_{20}H_{10}Cl_2O_5 = 401.19$

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*, and in *methyl alcohol*.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

2,6-Dichlorophenolindophenol Sodium Salt : Sodium salt of 2,6-dichloro-4-(4-hydroxyphenylamino)-cyclohexa-2,5-dien-1-one; Tillman's Reagent; $C_6H_2Cl_2{:}N{:}C_6H_4{\cdot}ONa,2H_2O = 326.11$.

Contains not less than 90.0 per cent of $C_{12}H_6O_2NCl_2Na,2H_2O$

DESCRIPTION – Dark green powder.

SOLUBILITY – Soluble in *water*, yielding not more than a trace of insoluble matter.

ASSAY – Dissolve about 0.3 g, accurately weighed, in 100 ml of *water*, add 3 g of *potassium iodide* and 25 ml of *dilute sulphuric acid*, and titrate with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.01631 g of $C_{12}H_6O_2NCl_2Na,2H_2O$.

2,6-Dichlorophenolindophenol Solution

Warm 0.1 g of *2,6-dichlorophenolindophenol sodium salt* with 100 ml of *water* and filter.

NOTE – 2,6-Dichlorophenolindophenol Solution must not be used later than 3 days after preparation.

2,6-Dichloroquinone Chlorimide : 2,6-Dichloro-*N*-chloro-*p*-benzoquinone Imine; $O{:}C_6H_2Cl_2{:}NCl = 210.45$

DESCRIPTION – Pale yellow crystalline powder.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol* and in dilute alkali hydroxide solutions.

MELTING RANGE–Between 65° and 67°, Appendix 5.11.

SOLUBILITY IN ALCOHOL – A solution of 0.1 g in 10 ml of *alcohol* is complete and clear.

RESIDUE ON IGNITION – Ignite 500 mg with 0.5 ml of *sulphuric acid*; the residue weighs not more than 1 mg.

SENSITIVITY – Complies with the requirements of the test for **SENSITIVITY** described under 2,6-dibromoquinone chlorimide.

Dicyandiamide : $NH_2{\cdot}C(:NH){\cdot}NH{\cdot}CN = 84.08$

Contains not less than 99.0 per cent of $C_2H_4N_4$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*.

MELTING RANGE – 209° to 212°, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.8 g and dissolve in 100 ml of *water*.

Transfer 10.0 ml to a 200 ml long-necked flask, add 10 ml of *nitrogen-free sulphuric acid* and evaporate by heating until fumes of sulphuric acid are evolved; complete the assay using the Method A for the *determination of nitrogen*, Appendix 3.3.5, beginning at the words 'Add 3 g of *anhydrous sodium sulphate* . . . . .' . Each ml of *0.1N sulphuric acid* is equivalent to 0.002102 g of $C_2H_4N_4$.

Diethylamine : $(C_2H_5)_2NH = 73.14$

Contains not less than 95.0 per cent $C_4H_{11}N$.

DESCRIPTION – Clear, colourless liquid.

REFRACTIVE INDEX – At 20°, 1.384 to 1.386, Appendix 5.14.

WT. PER ML – At 20°, 0.702 to 0.704 g, Appendix 5.19.

ASSAY – Add 3 g to 50 ml of *N sulphuric acid* and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.07314 g of $C_4H_{11}N$.

NN-Diethylaniline : $C_6H_5{\cdot}N(C_2H_5)_2 = 149.23$

DESCRIPTION – Colourless to pale yellow liquid with an ammoniacal odour.

SOLUBILITY – Slightly soluble in *water*, in *alcohol*, in *chloroform* and in *solvent ether*.

BOILING POINT – About 217°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.542 to 1.543, Appendix 5.14.

WT. PER ML – At 20°, about 0.93 g, Appendix 5.19.

Diethylene Glycol : Digol; $(CH_2OH{\cdot}CH_2)_2O = 106.12$.

DESCRIPTION – Clear, colourless liquid; practically odourless; taste, sharply sweetish; hygroscopic.

SOLUBILITY – Miscible with *water*, with *alcohol*, with *solvent ether*, and with *acetone*; insoluble in *benzene* and in *carbon tetrachloride*.

WT. PER ML – About 1.12 g, Appendix 5.19.

BOILING POINT – About 245°, Appendix 5.3.

Digitoxin Standard Solution

Dissolve 0.1250 g of *digitoxin R.S.* in sufficient *glacial acetic acid* to produce 100 ml. Dilute 4.0 ml of the solution with *glacial acetic acid* to 100 ml. To 25.0 ml of the resulting solution add 3.0 ml of *water* and mix well.

Digoxin Standard Solution

Dissolve 0.1250 g of *digoxin R.S.* in sufficient *glacial acetic acid* to produce 100 ml. Dilute 4.0 ml of this solution to 100 ml with *glacial acetic acid*. To 25.0 ml of the resulting solution add 3.0 ml of *water* and mix well.

Dimethylaminobenzaldehyde : *p*-Dimethylaminobenzaldehyde; 4-Dimethylaminobenzaldehyde
$(CH_3)_2.N.C_6H_4.CHO = 149.19$.

DESCRIPTION – White, or pale yellow, crystalline powder.

SOLUBILITY – Practically insoluble in *water*; freely soluble in *alcohol*.

MELTING RANGE – 73° to 75°, Appendix 5.11.

ORGANIC IMPURITIES – Dissolve 1 g in 20 ml of *dilute hydrochloric acid*; a clear solution free from any red colour is produced.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Dimethylaminobenzaldehyde Solution

Dissolve 0.125 g of *dimethylaminobenzaldehyde* in a cooled mixture of 65 ml of *sulphuric acid* and 35 ml of *water* and add 0.1 ml of *ferric chloride test solution*.

Dimethylaminobenzaldehyde Solution should be allowed to stand for twenty-four hours before use; it must be discarded when a yellow colour develops.

Dimethylaminobenzaldehyde Solution, Ethanolic

Dissolve 1 g of *dimethylaminobenzaldehyde* in 30 ml of *alcohol* and add 180 ml of *n-butyl alcohol* and 30 ml of *hydrochloric acid*.

Alcoholic Dimethylaminobenzaldehyde Solution should be freshly prepared; it must be discarded if a pink colour develops.

Dimethylaminobenzylidenerhodanine

$CO.NH.CS.S.C:CH.C_6H_4.N(CH_3)_2 = 264.36$

DESCRIPTION – Red powder.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol*; soluble in *acetone* forming a yellow solution.

SENSITIVITY – Add 0.5 ml of a 0.02 per cent w/v solution in *alcohol* to 15 ml of a 0.0005 per cent w/v solution of *silver nitrate* and add 1 g of *ammonium acetate*; a reddish colour is produced.

Dimethylaniline : *NN*-Dimethylaniline
$C_6H_5.N(CH_3)_2 = 121.18$

DESCRIPTION – Clear, oily liquid, almost colourless when freshly distilled, darkening to a reddish-brown colour on storage.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol*, in *chloroform*, in *solvent ether* and in dilute mineral acids.

SPECIFIC GRAVITY – About 0.960, Appendix 5.19.

DISTILLATION RANGE – Not less than 95 per cent distils between 192°and 194°, Appendix 5.3.

HYDROCARBONS – Dissolve 5 ml of a mixture of 15 ml of *dilute hydrochloric acid* and 10 ml of *water*. On cooling the solution to 10° no turbidity is produced.

ANILINE OR MONOMETHYLANILINE – Place 5 ml in a glass-stoppered flask, add 5 ml of a 1 in 10 solution of *acetic anhydride* in *benzene*, mix, and allow to stand for 30 minutes. Add 30.0 ml of *0.5N sodium hydroxide*, shake the mixture, add *phenolphthalein solution*, and titrate with *0.5N hydrochloric acid*. Perform a blank determination, and make necessary correction. Not more than 0.30 ml of *0.5 N sodium hydroxide* is consumed.

Dimethylformamide : $H.CO.N(CH_3)_2 = 73.09$

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

REFRACTIVE INDEX – At 20°, 1.430 to 1.432, Appendix 5.14.

WT. PER ML – At 20°, 0.948 to 0.951 g, Appendix 5.19.

Dimethylglyoxime : Diacetyldioxime
$CH_3.C(:NOH).C(:NOH).CH_3 = 116.12$

Contains not less than 99.0 per cent of $C_4H_8N_2O_2$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *alcohol*.

MELTING RANGE – 238° to 240°, with decomposition, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Dissolve 0.2 g in 20 ml of warm *alcohol* and add a boiling solution of 0.3 g of *nickel chloride* in 180 ml of *water*; add a solution of 1 g of *sodium acetate* in 10 ml of *water* and allow to stand for one hour. Filter, wash the precipitate with *water* containing one drop of *dilute ammonia solution*, and dry to constant weight at 105°. Each g of residue is equivalent to 0.803 g of $C_4H_8N_2O_2$.

Dimethylglyoxime Solution

A 1.0 per cent w/v solution of *dimethylglyoxime* in *alcohol*.

Dimethyl Sulphoxide : $(CH_3)_2SO = 78.13$

DESCRIPTION – Clear, colourless, viscous liquid; odourless or with a slight, but unpleasant odour; hygroscopic.

SOLUBILITY – Soluble in *water*, in *alcohol* and in *solvent ether*.

BOILING RANGE – Between 189° to 192°, Appendix 5.3.

WT. PER ML – At 20°, 1.100 to 1.103 g, Appendix 5.19.

Dinitrobenzoic Acid : 3,5-Dinitrobenzoic acid $(NO_2)_2C_6H_3.COOH = 212.12$

DESCRIPTION – Practically colourless crystals.

SOLUBILITY – Slightly soluble in *water*, very soluble in *alcohol*, and in *glacial acetic acid*; sparingly soluble in *solvent ether*, in *carbon disulphide* and in *benzene*.

MELTING RANGE – 205° to 207°, Appendix 5.11.

Dinitrobenzoic Acid Solution

A 2.0 per cent w/v solution of *dinitrobenzoic acid* in *alcohol*.

Dinitrobenzoyl Chloride : 3,5-Dinitrobenzoyl Chloride; $(NO_2)_2C_6H_3.COCl = 230.57$

Contains not less than 98.0 per cent of $C_7H_3O_5N_2Cl$, as determined by both parts of the **ASSAY** described below.

DESCRIPTION – Yellow, crystalline needles, decomposing in moist air.

SOLUBILITY – Completely soluble with decomposition in *sodium hydroxide solution*.

MELTING RANGE – 68° to 69°, Appendix 5.11.

PHOSPHORUS COMPOUNDS – Boil 1 g with 1 ml of *water* and 2 ml of *nitric acid* for one minute, cool, and filter; to the filtrate add 10 ml of *ammonium nitromolybdate solution* and warm at about 40° for two hours; no yellow precipitate is produced.

SULPHUR COMPOUNDS – Boil 1 g with 1 ml of *water* and 2 ml of *nitric acid* for one minute, add 25 ml of *water*, and filter; to the filtrate add 1 ml of *barium chloride solution* and allow to stand for one hour; no turbidity is produced.

ASSAY – Accurately weigh about 0.5 g and dissolve in 20 ml of *pyridine*, add slowly 20 ml of *water*, and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01153 g of $C_7H_3O_5N_2Cl$. To the neutralised solution add 30 ml of *0.1N silver nitrate* and 5 ml of *nitric acid*, filter, wash and titrate the combined filtrate and washing with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.02306 g of $C_7H_3O_5N_2Cl$.

Dinitrophenylhydrazine : 2,4-Dinitrophenylhydrazine; $(NO_2)_2{\cdot}C_6H_3{\cdot}NH{\cdot}NH_2 = 198.14$

DESCRIPTION – Orange-red crystals or a crystalline powder.

SOLUBILITY – Practically insoluble in *water*; slightly soluble in *alcohol*.

CLARITY AND COLOUR OF SOLUTION – 0.5 g yields a clear yellow solution on heating with a mixture of 25 ml of *water*, and 25 ml of *hydrochloric acid*.

MELTING RANGE – 197° to 200°, with decomposition, Appendix 5.11.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

Dinitrophenylhydrazine Solution

Dissolve 1.5 g of *dinitrophenylhydrazine* in 20 ml of *sulphuric acid (50 per cent v/v)*. Dilute to 100 ml with *water* and filter.

Dinitrophenylhydrazine Solution must be freshly prepared.

Dioctyl Sodium Sulphosuccinate

$C_8H_{17}O_2C.CH.SO_3Na.CH_2.CO_2.C_8H_{17} = 444.56$.

Contains not less than 90.0 per cent of $C_{20}H_{37}NaO_7S$.

DESCRIPTION – White, waxy flakes; hygroscopic.

SOLUBILITY – Soluble in warm *water* and in *alcohol*.

WATER – Not more than 1.0 per cent w/w, Appendix 3.3.25.

ACIDITY – Dissolve 10 g in 100 ml of *alcohol (50 per cent)*, previously neutralised to *phenolphthalein solution* with *0.1N Sodium hydroxide*, and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator; not more than 3.7 ml is required.

ASSAY – Carry out the method for the *determination of esters*, Appendix 3.3.2, using about 3 g, accurately

weighed, and 50 ml of *0.5N alcoholic potassium hydroxide*. Each ml of *0.5N alcoholic potassium hydroxide* is equivalent to 0.1111g of $C_{20}H_{37}NaO_7S$.

Dioctyl Sodium Sulphosuccinate Solution

Add 7.5 ml of a 60 per cent w/v solution of *dioctyl sodium sulphosuccinate* in *alcohol* to 150 ml of *water* and warm to dissolve. Cool to room temperature and dilute with *water* to 1000 ml.

Dioctyl Sodium Sulphosuccinate Solution should be protected from light.

Dioxan : 1,4-Dioxan; Diethylene Dioxide;

$CH_2{\cdot}CH_2O{\cdot}CH_2{\cdot}CH_2O = 88.11$

DESCRIPTION – Colourless liquid; odour, ethereal; hygroscopic.

SOLUBILITY – Miscible with *water*, with *alcohol*, and with *solvent ether*.

DISTILLATION RANGE – Not less than 95.0 per cent distils between 101° and 103°, Appendix 5.3.

MELTING POINT – Solidifies when cooled in ice and does not completely remelt at temperatures below 10°, Appendix 5.11.

WT. PER ML – At 25°, about 1.031 g, Appendix 5.19.

PEROXIDE – Add 5 ml to a mixture of 1 g of *potassium iodide* dissolved in 10 ml of *water*, 5 ml of *dilute hydrochloric acid* and 2 ml of *starch solution* and mix; not more than a faint blue or borwn colour is produced.

NON-VOLATILE MATTER – When evaporated on a water-bath, and dried to constant weight at 105°, leaves not more than 0.01 per cent of residue.

NOTE – It is dangerous to determine the distillation range or the non-volatile matter on a sample which does not comply with the test for peroxide.

Diphenylamine : $(C_6H_5)_2NH = 169.23$

DESCRIPTION – White crystals; odour, characteristic.

SOLUBILITY – Practically insoluble in *water*; freely soluble in *alcohol*.

MELTING RANGE – 54° to 55°, Appendix 5.11.

NITRATE – Add 1 mg to a solution prepared by mixing 2 ml of *water* with 6 ml of *nitrogen-free sulphuric acid*, cooling to about 60° and adding 1 drop of *hydrochloric acid*; not more than the faintest blue colour is produced. On the addition of a trace of *dilute nitric acid*, a deep blue colour is produced.

Diphenylamine Solution

A 1.0 per cent w/v solution of *diphenylamine* in *nitrogen-free sulphuric acid*.

Diphenylamine Solution must be colourless and should be stored protected from light.

Diphenylbenzidine : $(C_6H_5{\cdot}NH{\cdot}C_6H_4)_2 = 336.42$

DESCRIPTION – White or faintly grey-coloured, crystalline powder.

MELTING RANGE – 246° to 250°, Appendix 5.11.

NITRATE – Dissolve 8 mg in a cooled mixture of 45 ml of *nitrogen-free sulphuric acid* and 5 ml of *water*; the solution is colourless or not more than very pale blue.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Diphenylcarbazide : 1,5-Diphenylcarbazide;
$(C_6H_5{\cdot}NH{\cdot}NH)_2CO = 242.27$

DESCRIPTION – White, crystalline powder which gradually acquires a pink tint on exposure to air.

SOLUBILITY – Practically insoluble in *water*; soluble in *alcohol*.

Diphenylcarbazide Solution

A 0.2 per cent w/v solution of *diphenylcarbazide* in a mixture of 10 ml of *glacial acetic acid* and 90 ml of *alcohol (90 per cent)*.

SENSITIVITY TO CHROMATE – Dilute 0.5 ml of *0.1N potassium dichromate* to 1000 ml with *water*. Dilute 5 ml of this solution to 50 ml with *water*, add a few drops of *dilute hydrochloric acid* and 0.5 ml of *diphenylcarbazide solution*; a reddish-violet colour is produced, indicating a sensitivity of 1 part in 5 million.

Diphenylcarbazone : 1,5-Diphenylcarbazone;
$C_6H_5{\cdot}N{:}N{\cdot}CO{\cdot}NH{\cdot}NH{\cdot}C_6H_5 = 240.26$

DESCRIPTION – Orange, crystalline powder.

SOLUBILITY – Practically insoluble in *water*; soluble in *alcohol*.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

SENSITIVITY TO MERCURY – Add 0.1 ml of a 1 per cent w/v solution in *alcohol* to 10 ml of a solution containing the equivalent of 0.01 mg of mercury, Hg. A violet colour is apparent when compared with a solution containing no mercury.

Diphenylthiocarbazone : Dithizone; 1,5-Diphenylthiocarbazone; $C_6H_5{\cdot}N{:}NCS{\cdot}NH{\cdot}NH{\cdot}C_6H_5 = 256.32$

DESCRIPTION – Almost black powder.

SOLUBILITY – Practically insoluble in *water*; soluble in *chloroform*, in *carbon tetrachloride* and in other organic solvents, yielding solutions of an intense green colour.

LEAD – Shake 5 ml of a 0.1 per cent w/v solution in *chloroform* with a mixture of 5 ml of *water*, 2 ml of *lead-free potassium cyanide solution* and 5 ml of *strong ammonia solution*; the chloroform layer may remain yellow but has no red tint.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

Dipotassium Hydrogen Phosphate : Dibasic Potassium Phosphate; $K_2HPO_4 = 174.18$

Contains not less than 98.0 per cent of K_2HPO_4, calculated with reference to the substance dried at 105°.

DESCRIPTION – White granules or powder; somewhat hygroscopic.

SOLUBILITY – Very soluble in *water*.

ALKALINITY – Dissolve 2 g in 50 ml of *water*, boil for five minutes, cool to about 25° and add three drops of *phenolphthalein solution*; a red colour is produced which disappears on the addition of 1 ml of *N hydrochloric acid*.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 1 g complies with the *limit test for sulphates*, Appendix 3.2.8.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 5.0 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 3 g and dissolve in 100 ml of *water*, and titrate with *0.5N sulphuric acid*, using *bromocresol green solution* as indicator, and titrating to green colour indicative of pH 4.5. Each ml of *0.5N sulphuric acid* is equivalent to 0.08709 g of K_2HPO_4.

STORAGE – Store Dipotassium Hydrogen Phosphate in tightly closed containers.

Disodium Ethylenediaminetetraacetate

Edetate Disodium of the Indian Pharmacopoeia.

Disodium Hydrogen Arsenate : $Na_2HAsO_4,7H_2O = 312.02$

DESCRIPTION – Odourless crystals.

SOLUBILITY – Freely soluble in *water*; soluble in *glycerin*; slightly soluble in *alcohol*.

MELTING POINT – Melts at 57° when rapidly heated, Appendix 5.11.

Disodium Hydrogen Phosphate

Sodium Phosphate of the Indian Pharmacopoeia.

Disodium Hydrogen Phosphate, 0.2 M

Dissolve 71.63 g of *disodium hydrogen phosphate* in sufficient *water* to produce 1000 ml.

Disodium Hydrogen Phosphate, 0.25 M

Dissolve 89.53 g of *disodium hydrogen phosphate* in sufficient water to produce 1000 ml.

Disodium Hydrogen Phosphate, 1 M

Dissolve 358.14 g of *disodium hydrogen phosphate* in sufficient *water* to produce 1000 ml.

Disodium Hydrogen Phosphate, Anhydrous : Anhydrous Sodium Phosphate; $Na_2HPO_4 = 141.96$

Contains not less than 99.5 per cent of Na_2HPO_4, calculated with reference to the substance dried at 120° for one hour.

DESCRIPTION – White, hygroscopic powder.

SOLUBILITY – Soluble in *water*; practically insoluble in *alcohol*.

ALKALINITY – pH of a 2.0 per cent w/v solution, 9.0 to 9.2, Appendix 5.10.

IRON – Boil 0.5 g with 5 ml of *water* and 2 ml of *hydrochloric acid*, cool, add one drop of *0.1N potassium permanganate*, and mix. Add 10 ml of *ammonium thiocyanate solution* and 10 ml of a mixture of equal volumes of *amyl alcohol* and *amyl acetate*, shake vigorously, and allow to separate. Any red colour produced in the upper layer is not deeper than that produced by treating 1 ml of *standard iron solution* in a similar manner.

CHLORIDE – 0.5 g, dissolved in *water* with the addition of 2 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.10 g, dissolved in *water* with the addition of 3 ml of *dilute hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

LOSS ON DRYING – Loses not more than 0.4 per cent of its weight, when dried at 120° for one hour, Appendix 5.8.

ASSAY – Carry out the **ASSAY** described under Sodium Phosphate, using about 2.5 g, accurately weighed.

Disodium Hydrogen Phosphate Solution

A 10.0 per cent w/v solution of *disodium hydrogen phosphate* in *water*.

Dithizone Solution

A 0.05 per cent w/v solution of *diphenylthiocarbazone* in *chloroform*.

Eosin : CI 45380; Acid Red 87; Tetrabromofluorescein Disodium Salt; $C_{20}H_6O_5Br_4Na_2 = 691.86$

DESCRIPTION – Red powder; dissolves in *water* to yield a yellow to purplish-red solution with a greenish-yellow fluorescence.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

CHLORIDE – Dissolve 50 mg in 25 ml of *water*, add 1 ml of *nitric acid*, and filter; the filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

LOSS ON DRYING – When dried at 110° for two hours, loses not more than 10.0 per cent of its weight, Appendix 5.8.

SULPHATED ASH – Not more than 24.0 per cent, calculated with reference to the substance dried at 110° for two hours, Appendix 3.2.7.

Eosin Solution

A 0.5 per cent w/v solution of *eosin* in *water*.

Ergocalciferol

Of the Indian Pharmacopoeia.

Ether, Anaesthetic

Of the Indian Pharmacopoeia.

Ether, Solvent : $(C_2H_5)_2O = 74.12$

Solvent ether is diethyl ether. It contains a suitable stabiliser in a proportion not greater than 0.002 per cent w/v.

DESCRIPTION – Complies with the **DESCRIPTION** given under Anaesthetic Ether.

SOLUBILITY – Complies with the requirements for **SOLUBILITY** stated under Anaesthetic Ether.

WT. PER ML – At 20°, 0.714 to 0.718 g, Appendix 5.19.

BOILING RANGE – Between 34° and 36°, Appendix 5.3.

CAUTION—It is dangerous to determine the boiling range, if the sample does not comply with the test for peroxides.

METHYL ALCOHOL – Complies with the test for **METHYL ALCOHOL** described under Anaesthetic Ether.

ACETONE AND ALDEHYDE – Shake 10 ml occasionally during two hours with 1 ml of *potassium hydroxide solution*, in a glass-stoppered cylinder of colourless glass protected from light; no colour is produced in either layer. If colour is produced, distil carefully the solvent ether in a fractionating column and repeat the test on the distillate. Alternatively remove the stabiliser by a suitable solvent like a very dilute alkali solution, and perform the test. No colour is produced in either layer.

PEROXIDES – Place in a stoppered tube of about 12 ml capacity and about 1.5 cm in diameter, 8 ml of a freshly prepared *potassium iodide and starch solution* and fill to the brim with the solvent ether being tested. Place the stopper in position so that no air bubble is enclosed, shake vigorously, and set aside in the dark for thirty minutes; if any yellow colour is produced, it is not deeper than that of 0.5 ml of *0.001 N iodine* diluted with 8 ml of *potassium iodide and starch solution*.

ACIDITY – Place 10 ml of *alcohol (80 per cent)* in a glass-stoppered flask, add 0.5 ml of *phenolphthalein solution* and just sufficient *0.02 N sodium hydroxide* to produce a pink colour which persists after gently shaking the mixture for 30 seconds. Add a further quantity of 0.4 ml of *0.02 N sodium hydroxide* and 25 ml of the solvent ether. Stopper the cylinder, mix and shake gently for thirty seconds; the pink colour is not discharged.

NON-VOLATILE MATTER – Complies with the test for **NON-VOLATILE MATTER** described under Anaesthetic Ether.

CAUTION – It is dangerous to perform this test if the sample does not comply with the test for **PEROXIDES**.

STORAGE – Preserve Solvent Ether in a well-closed container, in a cool place, protected from light.

Ethyl Acetate : $CH_3{\cdot}CO{\cdot}O.C_2H_5 = 88.11$

Contains not less than 98.0 per cent w/w of $C_4H_8O_2$.

DESCRIPTION – Colourless liquid; odour, characteristic.

SOLUBILITY – Soluble in *water*; miscible with *alcohol*, and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 74° and 79°, Appendix 5.3.

WT. PER ML – At 25°, 0.895 g to 0.898 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

ASSAY – Carry out the **ASSAY** described under Methyl Salicylate. Each ml of *0.5 N alcoholic potassium hydroxide* is equivalent to 0.04405 g of $C_4H_8O_2$.

Ethyl Alcohol : Absolute Alcohol; Absolute Ethanol. Of the Indian Pharmacopoeia.

Ethyl Alcohol, Aldehyde-free

Ethyl Alcohol which complies with the following additional test:

ALDEHYDE – To 25 ml in a 300-ml flask add 75 ml of *dinitrophenylhydrazine solution*, heat on a water-bath under a reflux condenser for twenty-four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of *sulphuric acid*, and allow to stand for twenty-four hours; no crystals are produced.

Ethyl Carbamate : Urethane; $NH_2{\cdot}CO_2{\cdot}C_2H_5 = 89.10$

DESCRIPTION – Colourless, prismatic needless or leaflets.

SOLUBILITY – Highly soluble in *water*, in *alcohol*, and in *chloroform*; freely soluble in *solvent ether*, and in *glycerin*.

MELTING POINT – About 49°, Appendix 5.11.

Ethyl Cyanoacetate : $CN{\cdot}CH_2{\cdot}CO_2{\cdot}C_2H_5 = 113.12$

Contains not less than 94.0 per cent of $C_5H_7NO_2$.

DESCRIPTION – Colourless or pale brown liquid.

SOLUBILITY – Practically insoluble in *water*, miscible with *alcohol*, and with *solvent ether*.

WT. PER ML – At 20°, 1.058 to 1.063 g, Appendix 5.19.

FREE ACID – To 50 ml of *water*, add 0.5 g of *potassium iodate*, 2 g of *potassium iodide*, and 2 ml of *starch solution*; add 1 ml of the sample and titrate with *0.1N sodium thiosulphate*; not more than 0.4 ml of *0.1N sodium thiosulphate* is required.

ASSAY – Heat 0.4 g, accurately weighed, with a mixture of 2 ml of *sulphuric acid* and 15 ml of *water* under a reflux condenser for two hours. Cool, add 20 ml of *sodium hydroxide solution* and distil the liberated ammonia into 20 ml of *N hydrochloric acid*. Titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N hydrochloric acid* is equivalent to 0.1131 g of $C_5H_7NO_2$.

Ethylene Chloride : 1,2-Dichloroethane; Dichloroethane; $CH_2Cl{\cdot}CH_2Cl = 98.96$

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

SOLUBILITY – Freely soluble in *alcohol*, yielding a clear, colourless solution.

DISTILLATION RANGE – Not less than 95 per cent distils between 82° and 84°, Appendix 5.3.

WT. PER ML – At 20°, about 1.25 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.001 per cent w/v of residue.

Ethyl Iodide : $C_2H_5I = 155.97$

Contains not less than 99.0 per cent of C_2H_5I.

DESCRIPTION – A heavy, clear, refractive liquid; odour ethereal.

Colourless when freshly distilled, but rapidly becomes yellow to orange-brown.

SOLUBILITY – Slightly soluble in *water*; miscible with *alcohol* and with *solvent ether*.

REFRACTIVE INDEX – Between 1.513 and 1.514, Appendix 5.14.

WT. PER ML – Between 1.93 and 1.94 g, Appendix 5.19.

ASSAY – Boil 0.5 g with 25 ml of *alcoholic silver nitrate solution* for one hour under a reflux condenser. Cool in the dark, filter, wash the residue with *water*, and dry to constant weight at 105°. Each g of residue is equivalent to 0.6643 g of C_2H_5I.

Ethyl Iodide should be kept in a well-closed container, protected from light.

Ethyl Methyl Ketone : Butan-2-one; $C_2H_5COCH_3 = 72.10$

DESCRIPTION – Colourless, flammable liquid, with a characteristic acetone-like odour.

SOLUBILITY – Freely soluble in *water*, less soluble at higher temperatures; miscible with *alcohol*, with *solvent ether* and with *benzene*.

BOILING POINT – About 79°, Appendix 5.3.

WT. PER ML – About 0.81 g, Appendix 5.19.

Fast Yellow AB : CI 13015; Acid Yellow; E 105; $C_{12}H_9Na_2O_6S_2 = 401.34$

DESCRIPTION – An orange-yellow to red powder.

It complies with the following requirement:

HOMOGENEITY – Carry out the test for **SEPARATING POWER** described under Cellulose MN 300, applying to the plate a solution containing only the fast yellow AB; the chromatogram shows only one spot at an R_f of about 0.75.

Ferric Ammonium Sulphate : Ferric Alum $Fe(NH_4)(SO_4)_2,12H_2O = 482.18$

Contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $Fe(NH_4)(SO_4)_2,12H_2O$.

DESCRIPTION – Pale violet crystals, or a nearly colourless crystalline powder.

SOLUBILITY – Soluble in *water*, yielding a clear yellow or brown solution.

FERROUS ION – Dissolve 1 g in 50 ml of *water*, add 1 ml of *dilute hydrochloric acid* and 1 ml of *potassium ferricyanide solution*; no green or blue colour is produced.

ASSAY – Weigh accurately about 2 g, dissolve in 10 ml of *dilute hydrochloric acid* and dilute to 50 ml with *water*, add 3 g of *potassium iodide*, allow to stand for ten minutes and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator added towards the end of titration. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.04822 g of $Fe(NH_4)(SO_4)_2,12H_2O$.

Ferric Ammonium Sulphate Solution

A 8.0 per cent w/v solution of *ferric ammonium sulphate* in *water*.

Ferric Ammonium Sulphate Solution, Acid

Dissolve 0.2 g of *ferric ammonium sulphate* in 50 ml of *water* and add 6 ml of *dilute nitric acid* and sufficient *water* to produce 100 ml.

Ferric Chloride : Anhydrous Ferric Chloride; $FeCl_3$ = 162.22

DESCRIPTION – Greenish-black crystals or a crystalline powder, free from the orange colour of the hydrated salt, which is readily acquired by exposure to atmospheric moisture.

SOLUBILITY – Soluble in *water*, yielding an orange-coloured opalescent solution.

FERROUS SALTS – Dissolve 2.0 g in 100 ml of *water*, add 2 ml of *phosphoric acid* and titrate with *0.1N potassium permanganate* until a pink colour is produced; not more than 0.1 ml is required.

FREE CHLORINE – Dissolve 5 g in 10 ml of *water* and boil the solution; no blue colour is produced on a *starch-iodide paper* exposed to the vapours.

Ferric Chloride Hexahydrate : $FeCl_3,6H_2O = 270.32$

DESCRIPTION – Brownish-yellow or orange monoclinic crystals; very hygroscopic.

SOLUBILITY – Freely soluble in *water*, in *alcohol*, in *acetone*, and in *solvent ether*.

MELTING POINT – About 37°, Appendix 5.11.

Ferric Chloride Solution

Contains not less than 14.25 per cent and not more than 15.75 per cent w/v of $FeCl_3$.

DESCRIPTION – Clear, yellowish-brown liquid.

ASSAY – Dilute 2 ml with 20 ml of *water*, add 1 ml of *sulphuric acid* and *0.1N potassium permanganate* drop by drop until a pink colour persists for five seconds. Add 15 ml of *hydrochloric acid* and 2 g of *potassium iodide*, allow to stand for three minutes, and titrate with *0.1N sodium thiosulphate*, using *starch solution* as indicator added towards the end of titration. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.01622 g of $FeCl_3$.

Ferric Chloride Test Solution

A 5.0 per cent w/v solution of *ferric chloride* in *water*.

Ferrous Ammonium Sulphate : $Fe(NH_4)_2(SO_4)_2,6H_2O = 392.13$

Contains not less than 98.0 per cent of $Fe(NH_4)_2(SO_4)_2,6H_2O$.

DESCRIPTION – Pale greenish-blue crystals or crystalline powder.

SOLUBILITY – Soluble in freshly boiled and cooled *water*, yielding a yellowish-green solution.

ASSAY – Weigh accurately about 1.5 g and dissolve in 50 ml of freshly boiled and cooled *water*, add 2 ml of *phosphoric acid* and 20 ml of *dilute sulphuric acid* and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.03921 g of $Fe(NH_4)_2(SO_4)_2,6H_2O$.

Ferrous Sulphate

Of the Indian Pharmacopoeia.

Ferrous Sulphate Solution

A 2.0 per cent w/v solution of *ferrous sulphate* in freshly boiled and cooled *water*.

Ferrous Sulphate Solution must be freshly prepared.

Ferrous Sulphate Solution, Acid

A 0.45 per cent w/v solution of *ferrous sulphate* in freshly boiled and cooled *water* containing 0.5 ml of *hydrochloric acid*.

Ferrous Sulphate-Citrate Solution

Dissolve 1 g of *sodium metabisulphite* in 200 ml of *water*, add 1 ml of *N hydrochloric acid*, 1.5 g of *ferrous sulphate*, and 10 g of *sodium citrate*.

Ferrous Sulphate-Citrate Solution must be freshly prepared.

Ferrous Thiocyanate Reagent

Add 3 ml of *dilute sulphuric acid* to 35 ml of *water*, and boil to remove oxygen. To the hot solution add 1 g of *ferrous sulphate*, cool and add 0.5 g of *potassium thiocyanate*. Remove any pink colour by treating the solution with *reduced iron* and decant the solution from any excess of iron.

Ferrous Thiocyanate Reagent should be stored in an oxygen-free atmosphere and should not be used if any trace of pink colour is present.

Fibrin

The insoluble protein which separates during the coagulation of blood, washed and shredded.

Formaldehyde Solution : Formalin; $H{\cdot}CHO = 30.03$

Formaldehyde Solution is a solution of formaldehyde in *water* with *methyl alcohol* added to prevent polymerisation. It contains not less than 34.0 per cent w/w and not more than 38.0 per cent w/w of CH_2O.

DESCRIPTION – Colourless liquid; odour, characteristic, pungent and irritating; taste, burning. A slight white cloudy deposit is formed on long standing, especially in the cold, due to the separation of paraformaldehyde. This white deposit disappears on warming the solution.

SOLUBILITY – Miscible with *water*, and with *alcohol*.

ACIDITY – To 10 ml add 10 ml of *carbon dioxide-free water* and titrate with *0.1N sodium hydroxide*, using *bromothymol blue solution* as indicator; not more than 5 ml of *0.1N sodium hydroxide* is required.

WT. PER ML – At 20°, 1.079 to 1.094 g, Appendix 5.19.

ASSAY – Weigh accurately about 3 g and add to a mixture of 25 ml of *hydrogen peroxide solution* and 50 ml of *N sodium hydroxide*, warm on a water-bath until effervescence ceases and titrate the excess of alkali with *N sulphuric acid*, using *phenolphthalein solution* as indicator. Repeat the experiment with the same quantities of the same reagents in the same manner omitting the formaldehyde solution. The difference between the titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde. Each ml of *N sodium hydroxide* is equivalent to 0.03003 g of CH_2O.

STORAGE – Preserve Formaldehyde Solution in a well-closed container, preferably at a temperature not below 15°

Formaldehyde Solution, Dilute

Dilute 34 ml of *formaldehyde solution* with sufficient *water* to produce 100 ml.

Formamide : $H{\cdot}CO{\cdot}NH_2 = 45.04$

Contains not less than 98.0 per cent w/w of CH_3NO.

DESCRIPTION – Colourless, oily liquid; odour, slight.

SOLUBILITY – Soluble in *water* and in *alcohol*.

ASSAY – Weigh accurately about 1 g and distil with 50 ml of *N sodium hydroxide* in an ammonia distillation apparatus, into 40 ml of *N sulphuric acid*, and titrate the excess of sulphuric acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.04505 g of CH_3NO.

Formic Acid : $H{\cdot}CO_2H = 46.03$

Contains not less than 90.0 per cent w/w of CH_2O_2.

DESCRIPTION – Colourless liquid having a very pungent odour; highly corrosive.

SOLUBILITY – Miscible with *water*, and with *alcohol*.

WT. PER ML – At 20°, about 1.2 g, Appendix 5.19.

CHLORIDE – 1 ml complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 ml. Complies with the *limit test for sulphates*, Appendix 3.2.8.

NON-VOLATILE MATTER – When evaporated on a water bath, and dried to constant weight at 105°, leaves not more than 0.05 per cent w/v of residue.

ASSAY – Weigh a flask containing about 10 ml of *water*, quickly add about 1 ml of acid and reweigh, dilute with 50 ml of *water* and titrate with *N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.04603 g of CH_2O_2.

Formic Acid, Anhydrous : $HCO_2H = 46.03$

Contains not less than 98.0 per cent w/w of CH_2O_2.

DESCRIPTION – Colourless liquid; odour, pungent; highly corrosive.

SOLUBILITY – Miscible with *water*, and with *alcohol*.

WT. PER ML – At 20°, 1.218 to 1.221 g, Appendix 5.19.

CHLORIDE; SULPHATE; NON-VOLATILE MATTER – Complies with the tests described under Formic Acid.

ASSAY – Carry out the **ASSAY** described under Formic Acid.

D-Fructose : Laevulose; $C_6H_{12}O_6 = 180.16$

DESCRIPTION – White crystalline powder.

SOLUBILITY – Freely soluble in *water* and in hot *acetone*; soluble in *alcohol*, in *methyl alcohol* and in *pyridine*.

MELTING POINT – About 103°, with decomposition, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – At 20°, about −92°, determined on a 10 per cent w/v solution in *water* containing 0.05 ml of *5N ammonia*, Appendix 5.12.

Furfural : Furfuraldehyde; $C_4H_3O{\cdot}CHO = 96.09$

DESCRIPTION – Clear, colourless liquid when freshly distilled, but soon turns reddish brown.

SOLUBILITY – Soluble in *water*, and miscible with *alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 159° and 162°, Appendix 5.3.

STORAGE – Preserve Furfural in tight, light-resistant containers.

NOTE—Before use, it should be freshly distilled.

Gelatin

Of the Indian Pharmacopoeia.

D-Galactose : Cerebrose; $C_6H_{12}O_6 = 180.16$

DESCRIPTION – A white, crystalline powder.

SOLUBILITY – Very soluble in *water*; freely soluble in hot *water*; soluble in *pyridine*; and slightly soluble in *alcohol*.

MELTING POINT – About 164°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – At 20°, about +80°, determined on a 10 per cent w/v solution in *water*, Appendix 5.12.

D-Glucose

Dextrose (Anhydrous) of the Indian Pharmacopoeia.

Glucose Solution, Standard

Dissolve 0.100 g of D-*glucose* in a saturated solution of *benzoic acid* (approx. 0.25 per cent w/v) and dilute with a sufficient of the benzoic acid solution to produce 100.0 ml. Dilute 5.0 ml of the resulting solution with sufficient of the benzoic acid solution to produce 100.0 ml.

Standard Glucose Solution contains 0.05 mg of D-glucose per ml.

Glycerin : Glycerol

Of the Indian Pharmacopoeia.

Glyoxal Sodium Bisulphite : $[CH(OH)\cdot SO_3Na]_2,H_2O = 284.16$

Contains not less than 90.0 per cent of $C_2H_4Na_2O_8S_2,H_2O$.

DESCRIPTION – White or cream-coloured powder.

SOLUBILITY – Soluble in *water*.

ASSAY – Weigh accurately about 0.2 g and dissolve in 20 ml of *water*, add 50 ml of *0.1N iodine*, 2 g of *sodium bicarbonate* and titrate the excess of iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N iodine* is equivalent to 0.007104 g of $C_2H_4Na_2O_8S_2,H_2O$.

Guanine Hydrochloride : $C_5H_5N_5O,HCl,H_2O = 205.59$

DESCRIPTION – White crystalline powder.

SOLUBILITY – Slightly soluble in *water* and in *alcohol*; soluble in acidulated *water* and in *sodium hydroxide solution*.

MELTING POINT – Above 250°; with decomposition, Appendix 5.11.

SULPHATED ASH – Not more than 0.01 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 10.0 per cent, determined by drying in an oven at 105°, Appendix 5.8.

Hematoxylin : $C_{16}H_{14}O_6,3H_2O = 356.33$

DESCRIPTION – White to yellowish crystals.

SOLUBILITY – Slightly soluble in cold *water* and in *solvent ether*; soluble in hot *water*, in hot *alcohol*, in *glycerin* and in alkali hydroxides.

MELTING RANGE – Between 100° and 120°, Appendix 5.11.

Heparin

Heparin Sodium of the Indian Pharmacopoeia.

Heptane : *n*-Heptane; $C_7H_{16} = 100.20$

A fraction from petroleum containing about 85 per cent of *n*-heptane.

DESCRIPTION – Clear, colourless, volatile, flammable liquid.

SOLUBILITY – Insoluble in *water*; miscible with *alcohol*, with *solvent ether* and with *chloroform*.

DISTILLATION RANGE – Not less than 97 per cent distils between 97° and 98°, Appendix 5.3.

WT. PER ML – About 0.68 g, Appendix 5.19.

REFRACTIVE INDEX – At 20°, 1.378 to 1.379, Appendix 5.14.

CAUTION—May be irritating to respiratory tract, and in high concentration, narcotic.

Hexamine : $(CH_2)_6N_4 = 140.19$

Contains not less than 99.0 per cent of $C_6H_{12}N_4$.

DESCRIPTION – Colourless crystals or crystalline powder; odourless.

SOLUBILITY – Soluble in *water* and in *alcohol*.

ALKALINITY – A 10 per cent w/v solution is alkaline to *litmus solution*.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1.5 g and dissolve in 10 ml of *water*, add 50 ml of *N sulphuric acid*, and boil until the odour of formaldehyde is no longer perceptible. Titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.03505 g of $C_6H_{12}N_4$.

Hexamethyldisilazane : $(CH_3)_3\cdot Si\cdot NH\cdot Si\cdot (CH_3)_3 = 161.39$

DESCRIPTION – Clear, colourless liquid.

REFRACTIVE INDEX – At 20°, 1.406 to 1.409, Appendix 5.14.

WT. PER ML – At 20°, 0.773 to 0.775 g, Appendix 5.19.

Hexane : *n*-Hexane

A fraction from petroleum containing about 90 per cent of *n*-hexane.

DESCRIPTION – Colourless, mobile, highly flammable liquid.

DISTILLATION RANGE – Not less than 95 per cent, distils between 67° and 70°, Appendix 5.3.

WT. PER ML – At 20°, 0.670 to 0.677 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

Histamine Acid Phosphate

Of the Indian Pharmacopoeia.

Histamine Dihydrochloride : $C_5H_9N_3,2HCl = 184.07$

DESCRIPTION – White crystalline powder.

SOLUBILITY – Freely soluble in *water* and in *methyl alcohol*; soluble in *alcohol*.

MELTING POINT – About 250°, Appendix 5.11.

DL-Histidine Monohydrochloride

$N{:}CH.NH.CH{:}C.CH_2.CH(NH_2).COOH,HCl = 191.62$

Contains not less than 99.0 per cent of $C_6H_9N_3O_2,HCl$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*.

LOSS ON DRYING – Loses not more than 9.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Carry out the method for the *determination of nitrogen, Method A*, Appendix 3.3.5, using 0.15 g and 7 ml of *nitrogen-free sulphuric acid*. Each ml of *0.1N sulphuric acid* is equivalent to 0.00639 g of $C_6H_9N_3O_2,HCl$.

Holmium Oxide : $Ho_2O_3 = 377.86$

DESCRIPTION – A yellow solid.

SOLUBILITY – Practically insoluble in *water*.

Holmium Perchlorate Solution

A 5 per cent w/v solution of *holmium oxide* in *1.4 M perchloric acid*.

Hydrazine Hydrate : $NH_2.NH_2,H_2O = 50.06$

DESCRIPTION – Clear, colourless liquid with an ammoniacal odour.

SOLUBILITY – Miscible with *water*.

WT. PER ML – 1.03 g, Appendix 5.19.

Hydrazine Sulphate : $NH_2.NH_2,H_2SO_4 = 130.12$

Contains not less than 99.0 per cent of $N_2H_6SO_4$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Freely soluble in *water*; practically insoluble in *alcohol*.

MELTING POINT – About 254°, Appendix 5.11.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

IRON – 1 g complies with the *limit test for iron*, Appendix 3.2.5.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.1 g and dissolve in 20 ml of *water*. Add 3 g of *sodium bicarbonate* and titrate with *0.1N iodine*, using *starch solution* as indicator. Each ml of *0.1N iodine* is equivalent to 0.003253 g of $N_2H_6SO_4$.

Hydriodic Acid : HI = 127.91

Constant-boiling hydriodic acid contains 55.0 per cent w/w of HI (limits, 54.0 to 56.0).

DESCRIPTION – Almost colourless liquid when freshly made, but rapidly becoming yellow to brown owing to the liberation of iodine.

SOLUBILITY – Miscible in all proportions with *water* and with *alcohol*.

BOILING POINT – About 127°, Appendix 5.3.

WT. PER ML – At 20°, about 1.7 g, Appendix 5.19.

CHLORIDE AND BROMIDE – To 0.2 ml add 15 ml of *water*, 50 mg of *sodium sulphate*, 5 ml of *dilute ammonia solution* and 20 ml of *0.1N silver nitrate*, shake and filter; to the filtrate add 10 ml of *dilute nitric acid*. The opalescence produced is not greater than the standard opalescence obtained in the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Dilute 1 ml with 50 ml of *water* and add 1 ml of *barium chloride solution*. The turbidity produced should not be greater than the standard opalescence obtained in the *limit test for sulphates*, Appendix 3.2.8.

NON-VOLATILE MATTER – When evaporated on a water-bath, and dried to constant weight at 105°, leaves not more than 0.5 per cent w/w of residue.

ASSAY – Weigh accurately about 0.6 g into a stoppered flask containing about 10 ml of *water*, dilute with 25 ml of *water* and titrate the free iodine with *0.1N sodium thio-*

sulphate; then titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.01279 g of HI.

NOTE – If dark in colour, Hydriodic Acid should be redistilled over red phosphorus before use.

Hydrochloric Acid

Of the Indian Pharmacopoeia.

About 11.5 *N* in strength.

Hydrochloric Acid, *x*N

Solution of any normality *x*N may be prepared by diluting 85*x* ml of *hydrochloric acid* to 1000 ml with *water*.

Hydrochloric Acid (1 per cent w/v)

Dilute 1 g of *hydrochloric acid* to 100 ml with *water*.

Hydrochloric Acid, Dilute

Of the Indian Pharmacopoeia.

(Approximately 10 per cent w/w of HCl)

Hydrochloric Acid, Gaseous, Dry : Hydrogen Chloride; HCl = 36.46

Prepared by the action of *sulphuric acid* on *sodium chloride* and dried by passing over *sulphuric acid*.

Hydrochloric Acid, Iron-free

Hydrochloric acid which complies with the following additional test:

Evaporate 5 ml on a water-bath nearly to dryness add 40 ml of *water*, 2 ml of a 20 per cent w/v solution of *citric acid* and two drops of *thioglycollic acid*, mix, make alkaline with *dilute ammonia solution*, and dilute to 50 ml with *water*; no pink colour is produced.

Hydrochloric Acid, Methanolic, 0.1 N

To a 1000-ml volumetric flask containing 40 ml of *water* slowly add 8.6 ml of *hydrochloric acid*. Cool, and add *methyl alcohol* to volume.

Hydrochloric Acid, Methanolic, 0.5 N

To a 1000-ml volumetric flask containing 40 ml of *water* slowly add 43 ml of *hydrochloric acid*. Cool, and add *methyl alcohol* to volume.

Hydrocyanic Acid Solution

A solution containing 0.3 per cent w/v of HCN in *water*, which should be standardised immediately before use.

ASSAY – Place 50 ml in a flask containing 5 ml of *dilute ammonia solution*, add 0.25 ml of *potassium iodide solution* and titrate with *0.1N silver nitrate* until a permanent opalescence is obtained. Each ml of *0.1N silver nitrate* is equivalent to 0.005405 g of HCN.

Hydrofluoric Acid : HF = 20.01

A solution of hydrogen fluoride in *water*. Contains 40.0 per cent w/w of HF (limits, 39.0 to 43.0).

DESCRIPTION – Clear, colourless, corrosive liquid with a pungent odour.

RESIDUE ON IGNITION – When evaporated to dryness in a platinum dish and gently ignited to constant weight, leaves not more than 0.05 per cent w/w of residue.

ASSAY – Weigh accurately about 2 g into a flask containing 50 ml of *N sodium hydroxide*, and titrate the excess of alkali with *N sulphuric acid*, using *metacresol purple solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.02001 g of HF.

STORAGE – Hydrofluoric Acid should be stored in polyethylene or guttapercha bottles.

Hydrogen Peroxide Solution

Of the Indian Pharmacopoeia which complies with the following additional test:

CHLORIDE – To 10 ml add 40 ml of *water*, 1 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; no opalescence is produced.

Hydrogen Peroxide Solution, Strong

Contains 27.0 per cent w/w of H_2O_2 (limits, 26.0 to 28.0).

It may contain a suitable stabilising agent.

DESCRIPTION – Clear, colourless liquid; odour, faintly pungent.

ACIDITY – Dilute 10 ml with 100 ml of *water* and add 0.25 ml of *methyl red solution*; not less than 0.1 ml and not more than 0.5 ml of *0.1N sodium hydroxide* is required to change the colour of the solution.

CHLORIDE – 5 ml complies with the *limit test for chlorides*, Appendix 3.2.2.

STABILISING AGENT – Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts under reduced pressure at a temperature not exceeding 25°, and dry in a dessicator; any residue weighs not more than 10 mg.

NON-VOLATILE MATTER – When evaporated to dryness and gently ignited, leaves not more than 0.2 per cent w/v of residue.

ASSAY – Weigh accurately about 1 g and dilute to 100 ml with *water*. To 10 ml of the solution, add 40 ml of *dilute sulphuric acid*, and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.001701 g of H_2O_2.

STORAGE – Strong Hydrogen Peroxide Solution should be stored in a bottle closed with a glass-stopper or suitable plastic cap. It should be protected from light and stored in a cool place.

Hydrogen Peroxide Solution (30 per cent)

Contains 30.0 per cent w/w of H_2O_2 (limits, 29.0 to 31.0).

It may contain a suitable stabilising agent.

DESCRIPTION – Clear, colourless liquid; odour, faintly pungent.

ACIDITY; CHLORIDE; STABILISING AGENT; NON-VOLATILE MATTER – Complies with the tests described under Strong Hydrogen Peroxide Solution.

ASSAY – Carry out the **ASSAY** described under Strong Hydrogen Peroxide Solution.

STORAGE – As given under Strong Hydrogen Peroxide Solution.

Hydrogen Sulphide : $H_2S = 34.08$

Prepared by the action of *hydrochloric acid*, diluted with an equal volume of *water*, on iron sulphide; the resulting gas, is washed by passing through *water*.

DESCRIPTION – Colourless, poisonous gas, with a characteristic unpleasant odour.

Hydrogen Sulphide Solution

A recently prepared saturated solution of *hydrogen sulphide* in *water*.

Hydroquinone : 1,4-Dihydroxybenzene; Quinol; Benzene-1,4-diol; $C_6H_4(OH)_2 = 110.11$

DESCRIPTION – Colourless or almost colourless crystals or a crystalline powder.

SOLUBILITY – Soluble in *water*, yielding a clear colourless solution.

MELTING RANGE – 171° to 174°, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

8-Hydroxy-7-iodoquinoline-5-sulphonic acid : $C(OH):CI.CH:C(SO_3H).\overline{C:CN:CH.CH:CH}$; $C_9H_6INO_4S = 351.12$

DESCRIPTION – A yellow powder.

SOLUBILITY – Slightly soluble in cold *water*; more soluble in hot *water*.

Hydroxylamine Hydrochloride : Hydroxylammonium Chloride: $NH_2{\cdot}OH,HCl = 69.49$.

Contains not less than 97.0 per cent w/w of NH_2OH,HCl.

DESCRIPTION – Colourless crystals, or a white, crystalline powder.

SOLUBILITY – Very soluble in *water*; soluble in *alcohol*.

FREE ACID – Dissolve 1.0 g in 50 ml of *alcohol*, add 3 drops of *dimethyl yellow solution* and titrate to a full yellow colour with *N sodium hydroxide*; not more than 0.5 ml of *N sodium hydroxide* is required.

SULPHATED ASH – Not more than 0.2 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.1 g and dissolve in 20 ml of *water*, add 5 g of *ferric ammonium sulphate* dissolved in 20 ml of *water*, and 15 ml of *dilute sulphuric acid*, boil for five minutes, dilute with 200 ml of *water*, and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.003475 g of NH_2OH,HCl.

Hydroxylamine Hydrochloride, N

Dissolve 69.49 g of *hydroxylamine hydrochloride* in sufficient *water* to produce 1000 ml.

Hydroxylamine Hydrochloride Reagent in Alcohol (90 per cent)

Dissolve 70 g of *hydroxylamine hydrochloride* in 900 ml of *alcohol (90 per cent)*, add 4 ml of *dimethyl yellow solution*, and then add *N potassium hydroxide* in *alcohol (90 per cent)* until the full yellow colour of the indicator is produced. Add sufficient *alcohol (90 per cent)* to produce 1000 ml.

Hydroxylamine Hydrochloride Reagent in Alcohol (60 per cent)

Dissolve 34.75 g of *hydroxylamine hydrochloride* in 950 ml of *alcohol (60 per cent)*, add 5 ml of a 0.2 per cent w/v solution of *methyl orange* in *alcohol (60 per cent)* and then add *0.5 N potassium hydroxide* in *alcohol (60 per cent)* until the full yellow colour of the indicator is produced. Add sufficient *alcohol (60 per cent)* to produce 1000 ml.

The reagent complies with the following test:

To 10 ml add one drop of *0.5N potassium hydroxide* in *alcohol (60 per cent)*; no change in colour is produced. To a further 10 ml add one drop of *0.5 N hydrochloric acid*; the colour changes slightly towards orange.

Hydroxylamine Hydrochloride Solution

Dissolve 1 g of *hydroxylamine hydrochloride* in 50 ml of *water* and add 50 ml of *alcohol*, 1 ml of *bromophenol blue solution*, and *0.1N sodium hydroxide* until the solution becomes green.

Hypophosphorus Acid, Dilute

Contains 10 per cent w/w of H_3PO_2 (limits, 9.8 to 10.2).

DESCRIPTION – A clear, colourless liquid; odourless.

SOLUBILITY – Miscible with *water*, and with *alcohol*.

WT. PER ML – 1.037 to 1.039 g, Appendix 5.19.

BARIUM AND CALCIUM – Dilute 1.0 g with 10 ml of *water*, add 1 ml of *dilute sulphuric acid*, and allow to stand for one hour; no turbidity or precipitate is produced.

IRON – 0.20 g complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – Heat 1.0 g with 1 ml of *nitric acid* in a water-bath until the reaction ceases; the residue complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.50 g complies with the *limit test for sulphates*, Appendix 3.2.8.

PHOSPHORIC AND OXALIC ACIDS – Dilute 1.0 g with 10 ml of *water*, and add 1 ml of *calcium chloride solution* and 2 ml of *dilute ammonia solution*; not more than a slight turbidity is produced.

ASSAY – Dilute 10 g, accurately weighed, with 50 ml of *water*, and titrate with *0.5N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *0.5N sodium hydroxide* is equivalent to 0.03300 g of H_3PO_2.

Hypophosphorus Reagent

Dissolve, by heating gently, 10 g of *sodium hypophosphite* in 20 ml of *water* and dilute to 100 ml with *hydrochloric acid*.

Imidazole : Glyoxaline; $\overline{CH{:}CH{\cdot}N{:}CH{\cdot}NH}$ = 68.08

Contains not less than 99.0 per cent of $C_3H_4N_2$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

MELTING RANGE – 89° to 93°, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.3 g and dissolve in 50 ml of *water*. Titrate with *0.1N sulphuric acid*, using *bromocresol green solution* as indicator. Each ml of *0.1N sulphuric acid* is equivalent to 0.006808 g of $C_3H_4N_2$.

Imidazole, Recrystallised

Twice recrystallise 25 g of *imidazole* from 100 ml of *toluene*, cooling in an ice-bath with stirring. Finally wash with *solvent ether* and dry at room temperature under vacuum over *silica gel*.

The reagent complies with the following test :

LIGHT ABSORPTION – Absorbance of an 8 per cent w/v solution at 325 nm, not more than 0.10, Appendix 5.15 A.

Imidazole-Mercury Reagent

Dissolve 8.25 g of *recrystallised imidazole* in 60 ml of *water* and add 10 ml of *5N hydrochloric acid*. Stir the solution magnetically and add, dropwise, 10 ml of a 0.27 per cent w/v solution of *mercuric chloride*. If a cloudy solution results, discard, and prepare a further solution by adding the mercuric chloride solution more slowly. Adjust the pH to 6.80 ± 0.05 with *5N hydrochloric acid* (about 4 ml is required) and add sufficent *water* to produce 100 ml.

Indigo Carmine : CI 73015; Sodium Indigotindisulphonate; $C_{16}H_8N_2Na_2O_8S_2$ = 466.40

Contains not less than 90.0 per cent of $C_{16}H_8N_2Na_2O_8S_2$, calculated with reference to the dried substance.

DESCRIPTION – Blue powder or blue granules with a coppery lustre; almost odourless; taste, saline.

SOLUBILITY – Sparingly soluble in *water*; readily soluble in warm *water*; almost insoluble in *alcohol*. It is precipitated from a solution in *water* by *sodium chloride*.

ACIDITY OR ALKALINITY – Dissolve 1.0 g in 20 ml of hot *water*, add 5 g of *sodium chloride*, shake, cool, and filter; 10 ml of the filtrate, diluted with an equal volume of *water*, requires for neutralisation not more than 0.2 ml of *0.1N sodium hydroxide* or of *0.1N hydrochloric acid*, using *methyl red solution* as indicator.

INSOLUBLE MATTER – Dissolve 1.0 g in 100 ml of *water*, filter, and wash the insoluble matter with *water*; the residue, dried at 105°, weighs not more than 5 mg.

LOSS ON DRYING – Loses not more than 10.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

SULPHATED ASH – 30.0 to 40.0 per cent, calculated with reference to the dried substance.

ASSAY – Weigh accurately about 0.5 g, dissolve in 100 ml of hot *water* and 10 ml of *dilute sulphuric acid*, add 10 g of *sodium potassium tartrate*, boil, pass in *carbon dioxide*, and titrate with *0.1N titanous chloride* until the blue colour is discharged and the colour is yellow or orange. Each ml of *0.1N titanous chloride* is equivalent to 0.02332 g of $C_{16}H_8N_2Na_2O_8S_2$.

Indigo Carmine Solution

To a mixture of 10 ml of *hydrochloric acid* and 990 ml of a 20.0 per cent w/v solution of *nitrogen-free sulphuric acid* in *water*, add sufficient *indigo carmine* to produce a solution which complies with the following test:

Add 10 ml to a solution of 1.0 mg of *potassium nitrate* in 10 ml of *water*, add rapidly 20 ml of *nitrogen-free sulphuric acid* and heat to boiling; the blue colour is just discharged in one minute.

Indophenol Blue : CI 49700; $C_{18}H_{26}N_2O$ = 276.34

DESCRIPTION – Dark purple powder.

SOLUBILITY – Soluble in *alcohol*, and in *toluene*, giving dark purple solutions.

It complies with the following test:

HOMOGENEITY – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and *ethylene chloride* as the mobile phase. Apply to the plate 10 µl of a 0.01 per cent w/v solution in *ethylene chloride*. After removal of the plate allow it to dry in air; the chromatogram shows only one spot, but a stain may remain at the origin.

Iodine

Of the Indian Pharmacopoeia.

Iodine Solution

Dissolve 2.0 g of *iodine* and 3 g of *potassium iodide* in *water* to produce 100 ml.

Iodine Bromide : IBr = 206.81

DESCRIPTION – Blue-black or brownish-black crystals with lachrymatory vapours.

MELTING POINT – About 40°, Appendix 5.11.

BOILING POINT – About 116°, Appendix 5.3.

CAUTION – Iodine bromide vapours are corrosive to the eyes and mucous membranes.

Iodine Bromide Solution

Dissolve 20 g of *iodine bromide* in sufficient *glacial acetic acid* to produce 1000 ml.

STORAGE – Iodine Bromide Solution should be kept in a well-closed container and protected from light.

Iodine Monochloride Solution

Dissolve 8 g of *iodine trichloride* in about 200 ml of *glacial acetic acid*; dissolve 9 g of *iodine* in 300 ml of *carbon tetrachloride*; mix the two solutions, and dilute with *glacial acetic acid* to 1000 ml.

STORAGE – Iodine Monochloride Solution should be kept in a stoppered bottle, protected from light and stored at a temperature not exceeding 15°.

Iodine Pentoxide : I_2O_5 = 333.81

Contains not less than 98.0 per cent of I_2O_5.

DESCRIPTION – White or greyish-white granules.

IODIDE – Dissolve 2 g, dried at 200°, in *water*; a colourless solution is formed.

ASSAY – Weigh accurately about 0.1 g and dissolve in 50 ml of *water*, add 3 g of *potassium iodide* and 10 ml of *dilute hydrochloric acid*, and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.002782 g of I_2O_5.

Iodine Trichloride : ICl_3 = 233.36

Contains not less than 95.0 per cent of ICl_3.

DESCRIPTION – Reddish-orange crystals.

SOLUBILITY – Soluble in *glacial acetic acid*.

ASSAY – Weigh accurately about 0.2 g and dissolve in 50 ml of *water*, add 3 g of *potassium iodide* and 10 ml of *dilute hydrochloric acid*, and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.005832 g of ICl_3.

Iodine Water

Mix one volume of *0.1N iodine* with four volumes of *water*.

Iron-Phenol Solution

Dissolve 1.054 g of *ferrous ammonium sulphate* in 20 ml of *water*, and add 1 ml of *sulphuric acid* and 1 ml of *strong hydrogen peroxide solution*. Mix, heat until effervescence ceases, and dilute to 50 ml with *water*. To 3 ml of this solution, add, with cooling, sufficient *sulphuric acid* to produce 100 ml.

Distil *phenol*, rejecting the first 10 per cent and last 5 per cent, and, taking precautions to exclude moisture, collect the distillate in a glass-stoppered flask which has a capacity of twice the volume of phenol. Place the flask in ice-bath and allow the phenol to crystallise, breaking the surface to ensure complete crystallisation. Add to the phenol 1.13 times its weight of the iron-sulphuric acid solution prepared as directed in the preceding paragraph, stopper the flask, and allow to stand without cooling but with occasional shaking until the phenol is liquefied, shake the mixture vigorously until mixed. Allow to stand in the dark for 16 to 24 hours. To the mixture add 23.5 per cent of its weight of a solution prepared by mixing 100 volumes of *sulphuric acid* with 110 volumes of *water*, mix and transfer to dry, glass-stoppered bottles.

STORAGE – Iron-Phenol Solution should be protected from light and used within six months.

Iron, Reduced

Contains not less than 80.0 per cent of metallic iron, Fe.

DESCRIPTION – A fine, greyish-black powder, free from metallic lustre, and from gritty particles.

SOLUBILITY – Insoluble in *water*, and in *alcohol*; almost completely soluble in *dilute hydrochloric acid*.

ASSAY – Shake in a stoppered-flask for ten minutes about

0.25 g, accurately weighed, with a hot solution of 1.25 g of *copper sulphate* in 20 ml of *water*; filter rapidly, and wash the filter with *water*; acidify the mixed filtrate and washing with *sulphuric acid* and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.005585 g of Fe.

Iron Salicylate Solution

Dissolve 0.5 g of *ferric ammonium sulphate* in 250 ml of *water* containing 10 ml of *dilute sulphuric acid* and add sufficient *water* to produce 500 ml. To 100 ml add 50 ml of a 1.15 per cent w/v solution of *sodium salicylate*, 20 ml of *dilute acetic acid*, and 80 ml of a 13.6 per cent w/v solution of *sodium acetate*. Add sufficient *water* to produce 500 ml.

Iron Salicylate Solution should be recently prepared and kept in a well-closed container, protected from light.

Iron Solution, Standard

See *limit test for iron*, Appendix 3.2.5.

Isatin : $C_8H_5NO_2$ = 147.13

DESCRIPTION – Brick-red crystals or crystalline powder.

SOLUBILITY – Very slightly soluble in cold *water*; almost completely soluble in hot *water*; soluble in *alcohol*, and in *dilute ammonia solution*.

MELTING RANGE – 200° to 204°, Appendix 5.11.

SULPHATED ASH – Not more than 0.2 per cent, Appendix 3.2.7.

Isobutyl Acetate : $CH_3{\cdot}CO_2{\cdot}CH_2{\cdot}CH(CH_3)_2$ = 116.16

DESCRIPTION – Clear, colourless, mobile liquid.

SOLUBILITY – Slightly soluble in *water*; miscible with *alcohol* and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 116° and 120°, Appendix 5.3.

WT. PER ML – At 20°, 0.869 to 0.871 g, Appendix 5.19.

REFRACTIVE INDEX – At 20°, 1.390 to 1.391, Appendix 5.14.

Isobutyl Alcohol : 2-Methylpropan-1-ol;
$(CH_3)_2{\cdot}CH{\cdot}CH_2OH$ = 74.12.

DESCRIPTION – Clear, colourless or faintly yellow liquid; odour, characteristic.

SOLUBILITY – Soluble in *water*; miscible with *alcohol* and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 106° and 109°, Appendix 5.3.

WT. PER ML – At 25°, about 0.801 g, Appendix 5.19.

FLUORESCENCE – When viewed in screened ultra-violet light, shows not more than the faintest trace of fluorescence.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

Isobutyl Methyl Ketone : 4-Methylpentan-2-one;
$(CH_3)_2{\cdot}CH{\cdot}CH_2{\cdot}CO{\cdot}CH_3$ = 100.16

Contains not less than 98.0 per cent of $C_6H_{12}O$.

DESCRIPTION – Clear, colourless liquid; odour, characteristic.

DISTILLATION RANGE – Not less than 95 per cent distils between 114° and 117°, Appendix 5.3.

WT. PER ML – At 20°, about 0.80 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

ASSAY – Dissolve 14 g of *hydroxylamine hydrochloride* in 200 ml of *water* and neutralise to *bromophenol blue solution* with *N sodium hydroxide*. Weigh accurately about 4 g of the isobutyl methyl ketone and dissolve in half of this solution, add sufficient *aldehyde-free alcohol* to produce a homogeneous solution, and allow to stand for one hour. Add 20 ml of *light petroleum (boiling range 40° to 60°)* and titrate with *N sodium hydroxide* until the colour of the aqueous layer matches that of the reserved portion of the solution. Each ml of *N sodium hydroxide* is equivalent to 0.1002 g of $C_6H_{12}O$.

Isoniazid

Of the Indian Pharmacopoeia.

Isoniazid Solution

Dissolve 0.1 g of *isoniazid* in 150 ml of *methyl alcohol* and add 0.12 ml of *hydrochloric acid* and sufficient *methyl alcohol* to produce 200 ml.

Iso-octane : 2,2,4-Trimethylpentane; Trimethylpentane;
$(CH_3)_3{\cdot}C{\cdot}CH_2{\cdot}CH{\cdot}(CH_3)_2$ = 114.23

DESCRIPTION – Colourless liquid; flammable.

SOLUBILITY – Insoluble in *water*; soluble in *ethyl alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 98° and 100°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.391 to 1.393, Appendix 5.14.

WT. PER ML – At 20°, 0.690 to 0.694 g, Appendix 5.19.

Isopropyl Alcohol : Propan-2-ol; $(CH_3)_2CH.OH$ = 60.10.

DESCRIPTION – Clear, colourless liquid; odour, characteristic; taste, burning; flammable.

SOLUBILITY – Miscible with *water*, with *chloroform*, and with *solvent ether*.

ACIDITY OR ALKALINITY – To 25 ml add 25 ml of *carbon dioxide-free water* and boil gently for five minutes, cool with precautions to exclude carbon dioxide. The solution is not alkaline to *phenolphthalein solution* and requires not more than 0.06 ml of *0.1N sodium hydroxide* to make it alkaline.

DISTILLATION RANGE – Not less than 95 per cent distils between 81° and 83°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.377 to 1.378, Appendix 5.14.

WT. PER ML – At 25°, 0.7830 to 0.7870 g, Appendix 5.19.

ALDEHYDES AND KETONES – Mix in a cylinder 25 ml with 25 ml of *water* and 50 ml of *hydroxylamine hydrochloride solution*, allow to stand for five minutes, and titrate with *0.1N sodium hydroxide* until the colour matches that of 50 ml of *hydroxylamine hydrochloride solution* contained in a similar cylinder, each being viewed down the axis of the cylinder; not more than 2.0 ml of *0.1N sodium hydroxide* is required.

NON-VOLATILE MATTER – When evaporated to dryness on a water-bath and dried to constant weight at 105°, leaves not more than 0.001 per cent w/v of residue.

WATER-INSOLUBLE MATTER – Mix 1 ml with 19 ml of *water*; no opalescence is produced.

WATER – Not more than 0.50 per cent w/w, Appendix 5.19.

Isopropyl Myristate : $C_{17}H_{34}O_2$ = 270.45

DESCRIPTION – Clear, practically colourless oily liquid: odourless.

SOLUBILITY – Insoluble in *water*, in *glycerin* and in *propylene glycol*; soluble in *alcohol*, in *acetone*, in *chloroform*, in *ethyl acetate*, in *toluene* and in mineral oils.

REFRACTIVE INDEX – At 20°, between 1.432 and 1.436, Appendix 5.14.

SPECIFIC GRAVITY – At 25°, between 0.846 and 0.854, Appendix 5.19.

Isopropylamine : 2-Aminopropane; $CH_3.CH.(NH_2).CH_3$ = 59.11

DESCRIPTION – Colourless, volatile, flammable liquid having a strong odour of ammonia.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *solvent ether*.

WT. PER ML – At 20°, about 0.69 g, Appendix 5.19.

DISTILLATION RANGE – Not less than 95 per cent distils between 31° and 33°, Appendix 5.3.

REFRACTIVE INDEX – At 20° , between 1.374 and 1.376, Appendix 5.14.

Kerosene

A mixture of hydrocarbons, chiefly of the methane series, as available in commerce.

Kieselguhr

A neutral diatomaceous earth, purified by treating with *dilute hydrochloric acid*, washing with *water* and drying.

Kieselguhr G

Consists of Kieselguhr treated with *hydrochloric acid* and calcined, to which is added about 15 per cent w/w of *calcium sulphate hemihydrate* ($CaSO_4.\frac{1}{2}H_2O$).

DESCRIPTION – Fine, greyish-white powder, the grey colour becomes more pronounced on triturating the product with *water*. The average particle size is between 10 to 40 µm.

CALCIUM SULPHATE CONTENT – Determine by the method described under *silica gel G*.

ALKALINITY – Shake 1 g for 5 minutes with 10 ml of *carbon dioxide-free water*. The pH of the suspension is between 7.0 to 8.0, Appendix 5.10.

SEPARATING POWER – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, preparing plates using a slurry of the kieselguhr G with *0.02M sodium acetate* and using a mixture of 65 volumes of *ethyl acetate*, 23 volumes of *isopropyl alcohol* and 12 volumes of *water* as the mobile phase. Apply to the plate 5 µl of 0.01 per cent w/v solutions containing respectively *lactose*, *sucrose*, D-*glucose*, D-*fructose*, D-*galactose* in *pyridine*. Allow the mobile phase to ascend 14 cm. The migration time of the solvent is about 40 minutes. Dry the plate and spray with about 10 ml of *anisaldehyde solution*, again dry at 105° for 10 minutes. The chromatogram shows five well-defined spots without tailing and well separated from each other.

Kieselguhr H

Description : Fine, greyish-white powder, the grey colour becomes more pronounced on triturating the product with *water*. The average particle size is between 10 to 40 µm.

ALKALINITY – Shake 1 g for 5 minutes with 10 ml of

carbon dioxide-free water. The pH of the suspension is between 6.4 to 8.0, Appendix 5.10.

SEPARATING POWER – Complies with the test for **SEPARATING POWER** described under Kieselguhr G.

Kovac's Reagent

Dissolve 5 g of *4-dimethylaminobenzaldehyde* in 75 ml of *amyl alcohol* by warming on a water-bath at 50° to 55°, cool, and add 25 ml of *hydrochloric acid*.

Kovac's Reagent should be light yellow to light brown in colour, and should be stored at a temperature between 2° and 10°, protected from light.

Lactic Acid

Of the Indian Pharmacopoeia.

Lactophenol

Dissolve 20 g of *phenol* in a mixture of 20 g of *lactic acid*, 40 g of *glycerin*, and 20 ml of *water*.

Lactose

Of the Indian Pharmacopoeia.

Lanthanum Nitrate : $La(NO_3)_3,6H_2O$ = 433.02

Contains not less than 97.0 per cent of $La(NO_3)_3,6H_2O$.

DESCRIPTION – Colourless crystals; deliquescent.

SOLUBILITY – Freely soluble in *water*.

ASSAY – Weigh accurately about 0.75 g and dissolve in 25 ml of *water*, add 3 ml of *dilute nitric acid*, 3 g of *hexamine*, and 4 drops of *xylenol orange solution*, and titrate with *0.05M disodium ethylenediaminetetraacetate* until the solution becomes pure yellow in colour. If fading of the colour of the indicator occurs towards the end of the titration more *hexamine* should be added. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.02165 g of $La(NO_3)_3,6H_2O$.

Lanthanum Nitrate Solution

A 5.0 per cent w/v solution of *lanthanum nitrate* in *water*.

Lead Acetate : Sugar of Lead; $(CH_3CO_2)_2Pb,3H_2O$ = 379.33

Contains not less than 99.5 per cent and not more than the equivalent of 104.5 per cent of $C_4H_6O_4Pb,3H_2O$.

DESCRIPTION – Small, white, transparent, monoclinic prisms, or heavy, crystalline masses; odour, acetous; taste, sweet and astringent. Efflorescent in warm air. Becomes basic when heated.

SOLUBILITY – Freely soluble in *water*, and in *glycerin*; sparingly soluble in *alcohol*.

WATER-INSOLUBLE MATTER – Dissolve 1 g in 10 ml of recently boiled and cooled *water*, a solution is produced which is at most faintly opalescent and becomes clear on the addition of one drop of *acetic acid*.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

COPPER, IRON, SILVER, AND ZINC – Dissolve 0.5 g in 10 ml of *water*, add 2 ml of *dilute sulphuric acid*, allow to stand for thirty minutes, and filter; to the filtrate add an excess of *potassium ferrocyanide solution*; no precipitate or colour is produced.

ASSAY – Weigh accurately about 0.8 g and dissolve in a mixture of 100 ml of *water* and 2 ml of *acetic acid*, add 5 g of *hexamine*, and titrate with *0.05M disodium ethylenediaminetetraacetate*, using 0.2 ml of *xylenol orange solution* as indicator, until the solution becomes pale bright yellow. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.01897 g of $C_4H_6O_4Pb,3H_2O$.

STORAGE – Preserve Lead Acetate in a well-closed container.

Lead Acetate Solution

A 10.0 per cent w/v solution of *lead acetate* in *carbon dioxide-free water*.

Lead Dioxide : PbO_2 = 239.20

DESCRIPTION – Dark brown powder.

CHLORIDE, BROMIDE, AND IODIDE – Warm 2.0 g with 50 ml of *water* and 2 ml of *nitric acid*, cool, filter, and add to the filtrate 1 ml of *silver nitrate solution*; any opalescence produced is not greater than the *standard opalescence* obtained in the *limit test for chlorides*, Appendix 3.2.2.

Lead Monoxide : Litharge; PbO = 223.20

Contains not less than 99.0 per cent of PbO, calculated with reference to the substance ignited to constant weight.

DESCRIPTION – Pale orange, or pale brick-red, heavy scales or powder; odourless.

SOLUBILITY – Practically insoluble in *water* and in *alcohol*; soluble in *acetic acid*, in *dilute nitric acid* and in warm solutions of alkali hydroxides.

CARBONATE AND MOISTURE – Loses not more than 1.0 per cent of its weight, when ignited to constant weight at a temperature of 450° to 500°.

ASSAY – Weigh accurately about 0.5 g, previously ignited to constant weight at a temperature of 450° to 500°, and dissolve in a mixture of 10 ml of *water* and 3 ml of *acetic acid* with the aid of gentle heat. Cool to 20°, dilute to 100 ml with *water*, add 5 g of *hexamine* and titrate with

0.05M disodium ethylenediaminetetraacetate, using 0.2 ml of *xylenol orange solution* as indicator, until the solution becomes pale bright yellow. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.01116 g of PbO.

Lead Nitrate : $Pb(NO_3)_2$ = 331.21
Contains not less than 99.0 per cent of $Pb(NO_3)_2$

DESCRIPTION – Colourless or white crystals, or a white crystalline powder.

SOLUBILITY – Soluble in *water*, forming a clear, colourless solution.

ASSAY – Weigh accurately about 0.3 g and dissolve in 150 ml of *water*, add 5 ml of *dilute acetic acid*, heat to boiling, add a slight excess of *potassium chromate solution*, and boil gently until the precipitate becomes granular; collect the precipitate in a Gooch crucible, wash it with hot *water*, and dry to constant weight at 120°. Each g of residue is equivalent to 1.025 g of $Pb(NO_3)_2$.

Lead Solution, Standard

See *limit test for heavy metals*, Appendix 3.2.4.

Lead Subacetate Solution

Dissolve 40.0 g of *lead acetate* in 90 ml of *carbon dioxide-free water*. Adjust the pH to 7.5 with *10N sodium hydroxide*, centrifuge, and use the clear, supernatant solution. The solution remains clear when stored in a well closed container.

Lithium : Li = 6.93

DESCRIPTION – A soft metal whose freshly cut surface is silvery-grey. It rapidly tarnishes in air.

SOLUBILITY – Reacts violently with *water*, yielding hydrogen and a solution of lithium hydroxide; soluble in *methyl alcohol*, yielding hydrogen and a solution of lithium methoxide; insoluble in *solvent ether*, in *light petroleum (boiling range 40° to 60°)* and in *liquid paraffin*.

Lithium and Sodium Molybdophosphotungstate Solution

Dissolve 100 g of *sodium tungstate* and 25 g of *sodium molybdate* in 800 ml of *water* in a 1500-ml flask; add 50 ml of *phosphoric acid* and 100 ml of *hydrochloric acid*, connect to a reflux condenser and heat for ten hours. Cool, add 150 g of *lithium sulphate*, 50 ml of *water* and 4 to 6 drops of *bromine* and allow to stand for two hours. Remove the excess of bromine by boiling the mixture for fifteen minutes without the condenser. Cool, filter and dilute with *water* to produce 1000 ml.

STORAGE – Lithium and Sodium Molybdophosphotungstate Solution should be stored at a temperature not exceeding 4°, and must not be used later than four months after preparation. It has a golden yellow colour and must not be used if any trace of green colour is present.

Lithium Sulphate : Li_2SO_4,H_2O = 127.95

DESCRIPTION – Colourless crystals or a white, crystalline powder.

SOLUBILITY – Freely soluble in *water*; practically insoluble in *alcohol*.

ACIDITY OR ALKALINITY – A 10 per cent w/v solution in *carbon dioxide-free water* is neutral to *litmus paper*.

IRON – 2 g complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – 3.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

LOSS ON IGNITION – Loses not less than 14.0 per cent and not more than 15.0 per cent of its weight, when ignited to constant weight.

Locke-Ringer's Solution

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride	0.24 g
Magnesium chloride	0.2 g
Sodium bicarbonate	0.5 g
Dextrose	0.5 g
Water, recently distilled from hard-glass flask,	a sufficient quantity to make 1000 ml.

Prepare fresh each day. The constituents (except the dextrose and the sodium bicarbonate) may be made up in stock solutions and diluted as needed.

Magenta, Basic : CI 42510; Fuchsin; Rosaniline hydrochloride; $[(H_2N.C_6H_4)_2C:C_6H_3(CH_3):NH_2^+]Cl^-$ = 337.85
The hydrochloride of rosaniline of such a purity that when used in the preparation of Decolorised Solution of Magenta, a nearly colourless solution is obtained.

DESCRIPTION – Dark red powder, or green crystals with a metallic lustre.

SOLUBILITY – Soluble in *water*, giving a deep reddish-purple solution.

SULPHATED ASH – Not more than 5.0 per cent, Appendix 3.2.7.

Magenta Solution, Decolorised

Dissolve 1 g of *basic magenta* in 600 ml of *water* and cool in an ice-bath; add 20 g of *sodium sulphite* dissolved in 100 ml of *water*; cool in an ice-bath and add, slowly with

constant stirring, 10 ml of *hydrochloric acid*; dilute with *water* to 1000 ml.

If the resulting solution is turbid, it should be filtered and if brown in colour, it should be shaken with sufficient *decolorising charcoal* (0.2 to 0.3 g) to render it colourless and then filtered immediately. Occasionally it is necessary to add 2 to 3 ml of *hydrochloric acid*, followed by shaking, to remove the little residual pink colour. The solution resulting from any of the foregoing modifications should be allowed to stand overnight before use.

Decolorised Magenta Solution should be protected from light.

Magnesium Acetate : $(CH_3.COO)_2Mg,4H_2O$ = 214.46

Contains not less than 99.0 per cent of $C_4H_6MgO_4,4H_2O$.

DESCRIPTION – Colourless crystals; deliquescent.

SOLUBILITY – Freely soluble in *water* and in *alcohol*.

ALKALINITY – pH of a 5.0 per cent w/v solution, 8.2 to 8.8, Appendix 5.10.

ASSAY – Weigh accurately about 0.8 g and dissolve in 100 ml of *water*, add 10 ml of *strong ammonia-ammonium chloride solution* and 0.5 ml of *eriochrome black T solution*, and titrate at 40° with *0.1M disodium ethylenediaminetetraacetate* until the last trace of red colour disappears and the solution becomes pure blue. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02145 g of $C_4H_6MgO_4,4H_2O$.

Magnesium Nitrate : $Mg(NO_3)_2,6H_2O$ = 256.41

Contains not less than 98.0 per cent of $Mg(NO_3)_2.6H_2O$.

DESCRIPTION – Colourless crystals; deliquescent.

SOLUBILITY – Soluble in *water*, yielding a clear solution.

IRON – 0.5 g complies with the *limit test for iron*, Appendix 3.2.5.

LEAD – Not more than 10 parts per million, Appendix 3.2.6.

CHLORIDE – 8 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Ignite 2 g and dissolve the residue in *dilute hydrochloric acid*.

The solution complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Dissolve 0.5 g in 50 ml of *water*, add 10 ml of *strong ammonia-ammonium chloride solution*, and titrate with *0.05M disodium ethylenediaminetetraacetate*, using 0.1 g of *mordant black 11 mixture* as indicator. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.01282 g of $Mg(NO_3)_2,6H_2O$.

Magnesium Oxide, Light

Of the Indian Pharmacopoeia.

Magnesium Perchlorate : $Mg(ClO_4)_2$ = 223.23

DESCRIPTION – White granules.

PARTICLE SIZE – All the particles pass through a *sieve No. 14* and not more than 5 per cent through a *sieve No. 22*.

WATER – Not more than 14 per cent w/w, Appendix 3.3.25.

ACIDITY OR ALKALINITY – Shake 2 g with 20 ml of *carbon dioxide-free water*; the solution requires for neutralisation not more than 0.1 ml of either *0.1N hydrochloric acid* or *0.1N sodium hydroxide*.

Magnesium Powder : Mg = 24.31

Contains not less than 95.0 per cent of Mg.

DESCRIPTION – A coarse, dark silver-grey powder.

SOLUBILITY – Insoluble in *water* and in solutions of alkali hydroxides; soluble in dilute mineral acids.

ASSAY – Weigh accurately about 0.25 g and dissolve in 7 ml of *4N hydrochloric acid* and dilute to 250 ml with *water*. To 50 ml of this solution add 10 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05M disodium ethylenediaminetetraacetate*, using 0.1 g of *mordant black 11 mixture* as indicator. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.001215 g of Mg.

Magnesium Sulphate

Of the Indian Pharmacopoeia.

Magnesium Sulphate Solution, Ammoniacal

Dissolve 10 g of *magnesium sulphate* and 20 g of *ammonium chloride* in 80 ml of *water* and add 42 ml of *5N ammonia*. Allow to stand for a few days in a well-closed container, decant and filter.

Magnesium Uranyl Acetate Solution

Heat on a water-bath 3.2 g of *uranyl acetate*, 10 g of *magnesium acetate*, 2 ml of *glacial acetic acid* and 30 ml of *water*. When solution is complete allow to cool, add 50 ml of *alcohol* and dilute with *water* to 100 ml. Allow to stand for twenty-four hours, and filter.

Mallory's Stain

Dissolve 0.5 g of *water-soluble aniline blue*, 2.0 g of *orange G* and 2.0 g of *oxalic acid* in 100 ml of *water*.

Manganese Dioxide : MnO_2 = 86.94

Contains not less than 70.0 per cent of MnO_2.

DESCRIPTION – Black or brownish-black powder.

CHLORIDE – Warm 1 g with a mixture of 20 ml of *water*, 3 ml of *nitric acid* and 5 ml of *hydrogen peroxide solution* until solution is complete; cool and dilute to 50 ml with *water*; the solution complies with the *limit test for chlorides*, Appendix 3.2.2.

ASSAY – Place about 0.2 g, accurately weighed, in a stoppered flask, add 50 ml of *water*, 3 g of *potassium iodide* and 10 ml of *dilute hydrochloric acid*, and shake until solution is complete; titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator added towards the end of titration. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.004347 g of MnO_2.

Manganese Sulphate : $MnSO_4,H_2O = 169.01$

DESCRIPTION – Slightly rose-coloured crystals.

SOLUBILITY – Freely soluble in *water*, practically insoluble in *alcohol*.

Mercuric Acetate : $(CH_3CO_2)_2Hg = 318.68$

Contains not less than 98.0 per cent of $C_4H_6O_4Hg$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in warm *water*; hydrolyses on boiling.

ASSAY – Weigh accurately about 0.7 g and dissolve in a mixture of 40 ml of *water* and 5 ml of *dilute nitric acid*. Add 5 ml of *ferric ammonium sulphate solution* and titrate with *0.1N ammonium thiocyanate*. Each ml of *0.1N ammonium thiocyanate* is equivalent to 0.01593 g of $C_4H_6O_4Hg$.

Mercuric Acetate Solution

A 5.0 per cent w/v solution of *mercuric acetate* in *glacial acetic acid*.

Mercuric Chloride : $HgCl_2 = 271.50$

Contains not less than 99.5 per cent of $HgCl_2$.

DESCRIPTION – Heavy, colourless or white, crystalline masses, or a white crystalline powder.

SOLUBILITY – Soluble in *water*; freely soluble in *alcohol*.

NON-VOLATILE MATTER – When volatilised, leaves not more than 0.1 per cent of residue.

ASSAY – Weigh accurately about 0.3 g and dissolve in 85 ml of *water* in a stoppered-flask, add 10 ml of *calcium chloride solution*, 10 ml of *potassium iodide solution*, 3 ml of *formaldehyde solution* and 15 ml of *sodium hydroxide solution*, and shake continuously for two minutes. Add 20 ml of *acetic acid* and 35 ml of *0.1N iodine*, shake continuously for about ten minutes, or until the precipitated mercury is completely redissolved, and titrate the excess of iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N iodine* is equivalent to 0.01357 g of $HgCl_2$.

Mercuric Chloride, 0.2 M

Dissolve 54.30 g of *mercuric chloride* in sufficient *water* to produce 1000 ml.

Mercuric Chloride Solution

A 5.0 per cent w/v solution of *mercuric chloride* in *water*.

Mercuric Oxide, Nitrogen-free

Yellow mercuric oxide which complies with the following additional test, but does not necessarily comply with the requirement for content of HgO.

NITROGEN – Carry out the *determination of nitrogen*, Appendix 3.3.5, using 0.3 g of the sample, 3 g of *anhydrous sodium sulphate*, 0.1 g of *dextrose* and 7 ml of *nitrogen-free sulphuric acid*. The difference between the titrations is not more than 0.1 ml of *0.1N sulphuric acid*.

Mercuric Oxide, Yellow : $HgO = 216.59$

Contains not less than 99.0 per cent of HgO, calculated with reference to the substance dried at 105° for one hour.

DESCRIPTION – Orange-yellow, heavy, amorphous powder; odourless, stable in air but becomes discoloured on exposure to light.

SOLUBILITY – Practically insoluble in *water* and in *alcohol*; freely soluble in *dilute hydrochloric acid* and in *dilute nitric acid*, forming colourless solutions.

ACIDITY OR ALKALINITY – Shake 1 g with 5 ml of *water* and allow to settle; the supernatant liquid is neutral to *litmus solution*.

MERCUROUS SALTS – A solution of 0.5 g in 25 ml of *dilute hydrochloric acid* is not more than slightly turbid.

CHLORIDE – To 0.2 g add 1 g of *zinc powder* and 10 ml of *water*, shake occasionally during ten minutes and filter; the solution complies with the *limit test for chlorides*, Appendix 3.2.2.

LOSS ON DRYING – Loses not more than 1 per cent of its weight when dried at 105° for one hour, Appendix 5.8.

SULPHATED ASH – When moistened with *sulphuric acid* in a silica dish and heated strongly to constant weight, leaves not more than 0.5 per cent of residue.

ASSAY – Weigh accurately about 0.4 g, dissolve in 5 ml of *nitric acid* and 10 ml of *water*, and dilute with *water* to 150 ml. Titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Carry out the titration at a temperature not above 20°. Each

ml of *0.1N ammonium thiocyanate* is equivalent to 0.01083 g of HgO.

STORAGE – Preserve Yellow Mercuric Oxide in a well-closed container, protected from light.

Mercuric Sulphate : Mercury (II) Sulphate; $HgSO_4$ = 296.68

Contains not less than 99.0 per cent of $HgSO_4$.

DESCRIPTION – A white, crystalline powder. Hydrolyses in *water*.

SOLUBILITY – Soluble in *dilute sulphuric acid*.

CHLORIDE – Dissolve 2.0 g in a mixture of 2 ml of *dilute sulphuric acid* and 10 ml of *water*. Add 2 g of *zinc powder*, shake frequently for five minutes and filter. The filtrate complies with the *limit test for chlorides*, Appendix 3.2.2.

NITRATE – Dissolve 0.40 g in a mixture of 9 ml of *water* and 1 ml of *dilute sulphuric acid*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the blue colour is not entirely discharged.

ASSAY – Dissolve 0.6 g in a mixture of 10 ml of *dilute nitric acid* and 40 ml of *water*. Titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N ammonium thiocyanate* is equivalent to 0.01483 g of $HgSO_4$.

Mercuric Sulphate Solution

Mix 5 g of *yellow mercuric oxide* with 40 ml of *water*, and while stirring, add 20 ml of *sulphuric acid*, and 40 ml of *water*, and stir until completely dissolved.

Mercury : Hg = 200.59

Contains not less than 99.5 per cent of Hg.

DESCRIPTION – Shining, silvery-white, heavy liquid, easily divisible into globules and extremely mobile; readily volatilises on heating.

SOLUBILITY – Practically insoluble in *water*, in *alcohol* and in *hydrochloric acid*; readily and completely soluble in *nitric acid*, and boiling *sulphuric acid*.

WT. PER ML – At 25°, about 13.5 g, Appendix 5.19.

NON-VOLATILE MATTER – Leaves not more than 0.02 per cent w/w of residue, when volatilised at about 300°.

ASSAY – Weigh accurately about 0.4 g and dissolve in 20 ml of a mixture of equal parts of *water* and *nitric acid*, and heat gently until the solution is colourless. Add 150 ml of *water* and sufficient *potassium permanganate solution* to produce a permanent pink colour. Decolorise the pink colour by the addition of a trace of *ferrous sulphate* and titrate with *0.1N ammonium thiocyanate* at a temperature not exceeding 20°, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N ammonium thiocyanate* is equivalent to 0.01003 g of Hg.

Mercury Nitrate Solution : Million's Reagent

Dissolve 3 ml of *mercury* in 27 ml of cold *fuming nitric acid*; dilute the solution with an equal volume of *water*.

STORAGE – Mercury Nitrate Solution may be stored, protected from light for not more than two months.

Metaphosphoric Acid : $(HPO_3)_x$

DESCRIPTION – Glassy lumps or sticks containing a proportion of sodium metaphosphate; hygroscopic.

SOLUBILITY – Slowly soluble in *water*.

NITRATE – Boil 1 g with 10 ml of *water*, cool, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the blue colour is not entirely discharged.

REDUCING SUBSTANCES – Boil 1 g with 10 ml of *water* and add 2 drops of *0.1N potassium permanganate*; the pink colour is not entirely discharged.

Metaphosphoric-Acetic Acid Solution

Dissolve 15 g of *metaphosphoric acid* in 40 ml of *glacial acetic acid* and add *water* to produce 500 ml.

STORAGE – Store in a cool place and use within two days.

Methoxyethanol : 2-Methoxyethanol; Ethylene Glycol Monomethyl Ether; $CH_3O.CH_2.CH_2OH$ – 76.09

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 123° and 125°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.401 to 1.403, Appendix 5.14.

WT. PER ML – At 20°, 0.962 to 0.965 g, Appendix 5.19.

Methyl Alcohol : Methanol; CH_3OH = 32.04

DESCRIPTION – Clear, colourless liquid with a characteristic odour.

SOLUBILITY – Miscible with *water*, forming a clear colourless liquid.

SPECIFIC GRAVITY – At 25°, not more than 0.791, Appendix 5.19.

DISTILLATION RANGE – Not less than 95 per cent distils between 64.5° and 65.5°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.328 to 1.329, Appendix 5.14.

ACETONE – Place 1 ml in a Nessler cylinder, add 19 ml of *water*, 2 ml of a 1 per cent w/v solution of *2-nitrobenzaldehyde* in *alcohol (50 per cent)*, 1 ml of a 30 per cent w/v solution of *sodium hydroxide* and allow to stand in the dark for fifteen minutes. The colour developed does not exceed that produced by mixing 1 ml of *standard acetone solution*, 19 ml of *water*, 2 ml of the solution of *2-nitrobenzaldehyde* and 1 ml of the solution of *sodium hydroxide* and allowing to stand in the dark for fifteen minutes.

ACIDITY – To 5 ml add 5 ml of *carbon dioxide-free water*, and titrate with *0.1N sodium hydroxide*, using *bromothymol blue solution* as indicator; not more than 0.1 ml is required.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.005 per cent w/v of residue.

Methyl Alcohol, Dehydrated

Methyl alcohol which complies with the following additional requirements.

WATER – Not more than 0.1 per cent w/w, Appendix 5.3.25.

Methylamine Hydrochloride : $CH_3.NH_2,HCl = 67.52$

Contains not less than 97.0 per cent of CH_5N,HCl.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*.

ACIDITY – pH of a 10 pr cent w/v solution, 3.5 to 5.5, Appendix 5.10.

ASSAY – Weigh accurately about 0.3 g, dissolve in a mixture of 5 ml of *nitric acid* and 25 ml of *water*, add 50.0 ml of *0.1N silver nitrate* and 2 ml of *dibutyl phthalate*, shake for five minutes and titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.006752 g of CH_5N,HCl.

Methylaminophenol : 4-Methylaminophenol Sulphate; $(CH_3.NH.C_6H_4.OH)_2,H_2SO_4 = 344.39$

DESCRIPTION – White or cream-coloured, crystalline powder becoming darker on exposure to air.

SOLUBILITY – Slightly soluble in *water*.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Methylaminophenol with Sulphite Solution

Dissolve 0.1 g of *methylaminophenol*, 20 g of *sodium metabisulphite* and 1 g of *sodium sulphite* in sufficient quantity of *water* to produce 100 ml.

Methylaminophenol with Sulphite Solution, Strong

A solution in *water* containing 2 per cent w/v of *methylaminophenol* and 5 per cent w/v solution of *sodium metabisulphite*.

Methylamphetamine Hydrochloride

Of the Indian Pharmacopoeia.

Methylcellulose (4000 cps)

DESCRIPTION – White or creamy-white powder; odourless; tasteless.

SOLUBILITY – Swells in *water* to produce a clear to opalescent, viscous, colloidal suspension; insoluble in *alcohol*, in *solvent ether* and in *chloroform*; soluble in *glacial acetic acid*.

IDENTIFICATION – (1) Add 1 g to 100 ml of *water* and swirl to disperse the powder; to 10 ml of the solution add 0.5 ml of a 0.05 per cent w/v solution of *brilliant yellow* in *water*, 0.05 ml of *0.1N sodium hydroxide* and 10 ml of a saturated solution of *sodium sulphate*; a voluminous flocculent red precipitate is formed. Filter; the filtrate is colourless.

(2) Soak a few mg in *iodine water* for a few minutes, and remove the excess of reagent; the powder is stained yellow. Add one or two drops of a 66 per cent v/v solution of *sulphuric acid*; it is stained dark brown (distinction from microcrystalline cellulose).

pH – Between 6.0 and 8.0, determined in a 1 per cent w/v solution in *carbon dioxide-free water*, Appendix 5.10.

HEAVY METALS : Not more than 5 parts per million, determined by Method B on 4 g, Appendix 3.2.4.

SULPHATED ASH – Not more than 1.0 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for one hour, Appendix 5.8.

VISCOSITY – Between 3000 and 5000 centipoises, determined by Method B, Appendix 5.18, on a solution prepared in the following manner: Place 2.0 g, calculated with reference to the dried substance, in a wide-mouthed bottle, add 100 ml of *water* heated to about 90°, close the bottle with a stopper fitted with stirrer, and stir for ten minutes. Place the bottle in ice and continue the stirring until a solution of uniform consistency is obtained. Remove the ice and allow the solution to attain room temperature.

Express the result as dynamic viscosity in centipoises.

Methylene Blue : CI 52015; Tetramethylthionine Chloride; $C_{16}H_{18}ClN_3S,2H_2O = 355.90$

Contains not less than 96.0 per cent and not more than the equivalent of 101.0 per cent of $C_{16}H_{18}ClN_3S$, calculated

with reference to the substance dried to constant weight at 105°.

DESCRIPTION – Dark greenish, crystalline powder with a metallic lustre, or a dull, dark green, or brown powder; almost odourless; hygroscopic.

SOLUBILITY – Soluble in *water*; slightly soluble in *alcohol*, and in *chloroform*.

RESIDUE ON IGNITION – Ignite 1 g with 0.5 ml of *sulphuric acid*; the residue weighs not more than 10 mg.

LOSS ON DRYING – Loses not more than 15.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

ASSAY – Weigh accurately about 0.5 g and dissolve in 100 ml of *water*. Add 10 ml of *hydrochloric acid*, and heat to boiling. Replace the air in the flask with *carbon dioxide* and titrate with *0.1N titanous chloride* until the blue colour disappears, leaving the solution reddish-grey. Each ml of *0.1N titanous chloride* is equivalent to 0.01599 g of $C_{16}H_{18}ClN_3S$.

Methylene Chloride : Dichloromethane; $CH_2Cl_2 = 84.93$

DESCRIPTION – Clear, colourless, mobile liquid.

SOLUBILITY – Sparingly soluble in *water*; miscible with *alcohol* and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 39° and 41°, Appendix 5.3.

WT. PER ML – At 20°, 1.323 to 1.325 g, Appendix 5.19.

NON-VOLATILE MATTER – Leaves not more than 0.05 per cent w/v of residue, on evaporation on water-bath and drying at 105°.

Methyl Laurate : $CH_3.(CH_2)_{10}.CO_2.CH_3 = 214.35$

DESCRIPTION – Colourless or pale yellow liquid.

WT. PER ML – At 20°, about 0.87 g, Appendix 5.19.

Methyl Myristate : $CH_3.(CH_2)_{12}.CO_2.CH_3 = 242.40$

DESCRIPTION – Colourless or slightly yellow liquid.

WT. PER ML – At 20°, about 0.87 g, Appendix 5.19.

Methyl Palmitate : Methyl Hexadecanoate
$CH_3.(CH_2)_{14}.CO_2.CH_3 = 270.45$

DESCRIPTION – Colourless waxy solid.

CONGEALING TEMPERATURE – About 27°, Appendix 5.5.

WT. PER ML – At 30°, about 0.86 g, Appendix 5.19.

Methyl Stearate : $CH_3.(CH_2)_{16}.CO_2.CH_3 = 298.51$

DESCRIPTION – White or pale yellow, crystalline mass.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol* and in *solvent ether*.

MELTING POINT – About 38°, Appendix 5.11.

Molybdenum Trioxide : $MoO_3 = 143.94$

Contains not less than 99.0 per cent of MoO_3.

DESCRIPTION – Pale green powder.

AMMONIA-INSOLUBLE MATTER – Dissolve 5.0 g in 15 ml of *dilute ammonia solution*, filter through a tared filtering crucible, wash the residue with a mixture of 5 ml of *dilute ammonia solution* and 50 ml of *water*, reserving the filtrate for the test for **PHOSPHATE**, and dry at 110°; the residue weighs not more than 2.5 mg.

AMMONIUM SALTS – Dissolve 1.0 g in a mixture of 36 ml of *ammonia-free water* and 4 ml of *sodium hydroxide solution*; dilute 2 ml to 50 ml with *ammonia-free water*, and add 2 ml of *alkaline potassium mercuri-iodide solution*. Any colour produced is not deeper than that produced by adding 2 ml of *alkaline potassium mercuri-iodide solution* to 50 ml of *ammonia-free water* containing 1 ml of *dilute ammonium chloride solution (Nessler's)*.

PHOSPHATE – Pour the filtrate obtained in the test for **AMMONIA-INSOLUBLE MATTER** into 75 ml of *dilute nitric acid* and allow to stand at about 40° for two hours; no yellow precipitate is produced.

ASSAY – Activate a suitable zinc amalgam 'reductor' by passing through the column a mixture of 40 ml of *dilute sulphuric acid* and 60 ml of *water*, followed by 100 ml of *water*, pass through the column 200 ml of *N sulphuric acid* followed by 100 ml of *water* and collect in a receiver containing 25 ml of a solution containing 15 per cent w/v of *ferric ammonium sulphate* and 15 per cent v/v of *sulphuric acid*; add 5 ml of *phosphoric acid* to the receiver and titrate the mixture with *0.1 N potassium permanganate*. Reactivate the column with 100 ml of *N sulphuric acid*. Pass through the column a solution prepared by dissolving 0.1 g of the *molybdenum trioxide* in 60 ml of *water* and 10 ml of *dilute ammonia solution* and acidifying with 30 ml of *dilute sulphuric acid*, followed by 100 ml of *N sulphuric acid* and 100 ml of *water*. Collect in a receiver containing 25 ml of the acidified solution of *ferric ammonium sulphate* and 5 ml of *phosphoric acid*, and titrate the mixture with *0.1 N potassium permanganate*. The difference between the titrations represents the amount of potassium permanganate required by the molybdenum trioxide. Each ml of *0.1N potassium permanganate* is equivalent to 0.004798 g of MoO_3.

Molybdic Acid : $H_2MoO_4 = 161.95$

Contains not less than 85.0 per cent of MoO_3.

DESCRIPTION – White or pale cream-coloured powder containing ammonium acid molybdate.

SOLUBILITY – Soluble in *dilute ammonia solution*.

ASSAY – Dissolve 0.2 g, accurately weighed, in 40 ml of *dilute ammonia solution* and 50 ml of *water*, add 20 ml of *glacial acetic acid*, heat to boiling, and add 1 g of *lead acetate* dissolved in 50 ml of *water*; continue to boil until the precipitate becomes granular; collect the precipitate in a Gooch crucible, wash with boiling *water*, dry, and ignite to constant weight at a dull red heat. Each g of residue is equivalent to 0.3921 g of MoO_3.

Morphinated Water

Shake *morphine* with *chloroform water*, and allow to stand for not less than seven days at room temperature, shaking occasionally, in order to obtain a saturated solution of the alkaloid; filter from the undissolved morphine immediately before use.

Morphine

Add *dilute ammonia solution*, in slight excess, to a solution of *morphine sulphate* in *water*; wash the precipitated morphine with *water* until free from ammonium salts.

Morphine, Anhydrous

Morphine dried at 110°.

Morphine Hydrochloride

Of the Indian Pharmacopoeia.

Morphine Sulphate

Of the Indian Pharmacopoeia.

Morpholine : $\overline{NH.CH_2.CH_2.O.CH_2.CH_2}$ = 87.12

Contains not less than 99.0 per cent w/w of C_4H_9NO.

DESCRIPTION – Colourless liquid; odour, strong.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 126° and 130°, Appendix 5.3.

WT. PER ML – At 20°, 1.000 to 1.002 g, Appendix 5.19.

ASSAY – Weigh accurately about 3 g and dissolve in 40 ml of *N sulphuric acid* and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.08712 g of C_4H_9NO.

α-Naphthol : 1-Naphthol; $C_{10}H_7.OH$ = 144.17

DESCRIPTION – Colourless or white crystals or a white, crystalline powder; odour, characteristic.

SOLUBILITY – Freely soluble in *alcohol* yielding a not more than slightly opalescent, colourless or almost colourless solution, with no pink tint.

MELTING RANGE – 93° to 96°, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

α-Naphthol Solution : 1-Naphthol Solution

Dissolve 1 g of *α-naphthol* in a solution of 6 g of *sodium hydroxide* and 16 g of *anhydrous sodium carbonate* in 100 ml of *water*.

α-Naphthol Solution must be prepared immediately before use.

β-Naphthol : 2-Naphthol; $C_{10}H_7.OH$ = 144.17

DESCRIPTION – White leaflets or crystalline powder; odour, faint and resembling that of phenol.

SOLUBILITY – Very slightly soluble in *water*; soluble in *alcohol*, in *solvent ether*, in *chloroform*, and in solutions of alkali hydroxides.

ACIDITY OR ALKALINITY – Shake 1 g with 100 ml of *water* occasionally during fifteen minutes, and filter; the filtrate is neutral to the *litmus solution*.

MELTING RANGE – Between 121° and 123°, Appendix 5.11.

α-NAPHTHOL – Dissolve 0.1 g in 10 ml of boiling *water*, and add 1 ml of *ferric chloride test-solution*; a white precipitate is produced, which on heating becomes brown but not violet.

NAPHTHALENE AND OTHER ORGANIC SUBSTANCES – Dissolve 0.5 g in 25 ml of *dilute ammonia solution*; no residue remains, and the solution is not darker than pale yellow.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

β-Naphthol Solution

Dissolve 5 g of β-naphthol, freshly recrystallized, in 8 ml of *sodium hydroxide solution* and 20 ml of *water*, and add sufficient *water* to produce 100 ml.

β-Naphthol Solution must be freshly prepared.

α-Naphthylamine : 1-Naphthylamine; $C_{10}H_7.NH_2$ = 143.15

DESCRIPTION – White crystals, or crystalline lumps, becoming reddish on exposure to air; odour, unpleasant.

SOLUBILITY – Very slightly soluble in *water*; soluble in *alcohol*, and in *solvent ether*.

MELTING RANGE – 49° to 50°, Appendix 5.11.

STORAGE – 1-Naphthylamine should be kept in well-closed containers, protected from light.

Naphthylamine-Sulphanilic Acid Reagent

Immediately before use mix equal volumes of solutions A and B prepared as follows:

Solution A – Dissolve 0.5 g of *sulphanilic acid* in 30 ml of *6N acetic acid* and dilute to 150 ml with *water*.

Solution B – Dissolve 0.15 g of *α-naphthylamine* in 30 ml of *6N acetic acid* and dilute to 150 ml with *water*.

***N*-(1-Naphthyl)ethylenediamine Hydrochloride :** *N*-(1-Naphthyl)ethane-1,2-diammonium Dichloride $NH_2.CH_2.CH_2.NH(C_{10}H_7),2HCl$ = 259.18

Contains not less than 95.0 per cent of $C_{12}H_{14}N_2,2HCl$.

DESCRIPTION – White or cream coloured powder.

SOLUBILITY – Soluble in *water*.

ASSAY – Carry out the *determination of nitrogen, Method B*, Appendix 3.3.5, using about 0.25 g and 8 ml of *nitrogen-free sulphuric acid*. Each ml of *0.1N sulphuric acid* is equivalent to 0.01296 g of $C_{12}H_{14}N_2,2HCl$.

Niacin

Nicotinic Acid of the Indian Pharmacopoeia.

Nickel Chloride : $NiCl_2,6H_2O$ = 237.71

Contains not less than 98.0 per cent of $NiCl_2,6H_2O$.

DESCRIPTION – Apple-green crystals or crystalline powder.

SOLUBILITY – Soluble in *water*.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 1 g and dissolve in 100 ml of *water*, add a solution prepared by dissolving 3 g of *sodium acetate* in *water*, heat to 80°, and titrate with *0.1M disodium ethylenediaminetetraacetate*, using *mordant red 7 solution* as indicator. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02377 g of $NiCl_2,6H_2O$.

Nickel Solution, Standard

A 0.00401 per cent w/v solution of *nickel chloride* in *water*.

Standard Nickel Solution contains 0.01 mg of Ni in 1 ml.

Ninhydrin : Indanetrione Hydrate; $C_9H_4O_3,H_2O$ = 178.14

Contains not less than 96.0 per cent of $C_9H_4O_3,H_2O$.

DESCRIPTION – White or very pale yellow, crystalline powder.

SOLUBILITY – Soluble at 60° in 20 parts of *water*.

MELTING POINT – Not less than 250°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1 g and transfer to a stoppered-flask, add 40 ml of *hydroxylamine hydrochloride reagent in alcohol (90 per cent)*, and heat in a water-bath at a temperature of 70° to 85°; neutralise the liberated acid at five-minutes intervals with *N sodium hydroxide* and continue heating and neutralising until no more acid is liberated. Each ml of *N sodium hydroxide* is equivalent to 0.05938 g of $C_9H_4O_3,H_2O$.

Nitranilic Acid : 2,5-Dihydroxy-3,6-dinitro-1,4-benzoquinone; $CO.C(OH):C(NO_2).CO.C(OH):C(NO_2),2H_2O$ = 266.41

PREPARATION – Nitranilic acid may be prepared as follows:

Dissolve 20 g of *p-benzoquinone* in 150 ml of *fuming nitric acid* in a loosely stoppered-flask and allow to stand for several days at a temperature not exceeding 0°. Filter rapidly through a sintered glass filter, maintaining a temperature not exceeding 0°. Dry the precipitate of nitranilic acid by pressure between porous plates, and then dissolve it in the minimum amount of *water*, add about 15 ml of *nitric acid* for each 100 ml of solution or a sufficient quantity to reprecipitate the nitranilic acid. Collect the precipitate as before and dry over *sodium hydroxide* in vacuum at a pressure not exceeding 5 mm of mercury.

Contains not less than 65.0 per cent and not more than 86.0 per cent of $C_6H_2N_2O_8$.

DESCRIPTION – Orange or orange-yellow, crystalline powder.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

ASSAY – Weigh accurately about 0.3 g and dissolve in sufficient *water* to produce 250 ml. To 50 ml of this solution add 200 ml of *water* and 15 g of *potassium hydrogen tartrate*, and heat to boiling. Add 10 ml of *light green SF yellowish solution* and titrate with *0.1N titanous chloride* until the green colour is discharged. Repeat the operation without the sample; the difference between the titrations represents the amount of titanous chloride required. Each ml of *0.1N titanous chloride* is equivalent to 0.001644 g of $C_6H_2N_2O_8$.

Nitranilic Acid Solution

A 3.5 per cent w/v solution of *nitranilic acid* in *alcohol*.

Nitric Acid

Contains 70.0 per cent w/w of HNO_3 (limits, 69.0 to 71.0). About 16 N in strength.

DESCRIPTION – Clear, colourless, fuming liquid.

WT. PER ML – At 20°, 1.41 to 1.42 g, Appendix 5.19.

COPPPER AND ZINC – Dilute 1 ml with 20 ml of *water*, and add a slight excess of *dilute ammonia solution*; the mixture does not become blue. Pass *hydrogen sulphide*; a precipitate is not produced.

IRON – 0.5 ml complies with the *limit test for iron*, Appendix 3.2.5.

LEAD – Not more than 2 parts per million, Appendix 3.2.6.

CHLORIDE – 5 ml neutralised with *dilute ammonia solution*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – To 2.5 ml add 10 mg of *sodium bicarbonate* and evaporate to dryness on a water-bath; the residue, dissolved in *water*, complies with the *limit test for sulphates*, Appendix 3.2.8.

SULPHATED ASH – Not more than 0.01 per cent w/w, Appendix 3.2.7.

ASSAY – Weigh accurately about 4 g into a stoppered flask containing 40 ml of *water*, and titrate with *N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.06301 g of HNO_3.

Nitric Acid, *x*N

Solutions of any normality *x*N may be prepared by diluting 63*x* ml of *nitric acid* to 1000 ml with *water*.

Nitric Acid, Dilute

Contains approximately 10 per cent w/w of HNO_3.

Dilute 106 ml of *nitric acid* to 1000 ml with *water*.

Nitric Acid, Fuming : HNO_3 = 63.01

Contains not less than 95.0 per cent w/w of HNO_3.

DESCRIPTION – Clear, almost colourless to yellow, fuming liquid.

WT. PER ML – At 20°, about 1.5 g, Appendix 5.19.

COPPER AND ZINC; IRON; LEAD; CHLORIDE – Complies with the tests described under *nitric acid*.

SULPHATE – Dilute 0.25 ml with 5 ml of *water*, add 10 mg of *sodium bicarbonate*, and evaporate to dryness on a water-bath. The residue complies with the *limit test for sulphates*, Appendix 3.2.8.

RESIDUE ON IGNITION – When evaporated and gently ignited to constant weight, leaves not more than 0.01 per cent w/w of residue.

ASSAY – Carry out the **ASSAY** described under Nitric Acid, using about 3.0 g, accurately weighed.

Nitric Oxide : NO = 30.01

Nitric oxide, washed with *water*.

4-Nitroaniline : *p*-Nitroaniline; $NO_2.C_6H_4.NH_2$ = 138.13

DESCRIPTION – Deep yellow, crystalline powder.

MELTING RANGE – 146° to 148°, Appendix 5.11.

CLARITY OF SOLUTION – Heat 1 g with 25 ml of *dilute hydrochloric acid* and 25 ml of *water*; a clear yellow solution is obtained, which remains clear on cooling.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Nitroaniline Solution, Diazotised

Dissolve 0.4 g of *4-nitroaniline* in 60 ml of *N hydrochloric acid*, cool to 15°, and add a 10 per cent w/v solution of *sodium nitrite* in *water* until one drop of the mixture turns *starch-iodide paper* blue.

Diazotized Nitroaniline Solution must be freshly prepared.

2-Nitrobenzaldehyde : *o*-Nitrobenzaldehyde $NO_2.C_6H_4.CHO$ = 151.12

DESCRIPTION – Yellow needles; odour, resembling that of benzaldehyde.

SOLUBILITY – Soluble in *alcohol*.

MELTING RANGE – 40° to 45°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Nitrobenzene : $C_6H_5.NO_2$ = 123.11

DESCRIPTION – Pale yellow liquid; odour, characteristic.

SOLUBILITY – Insoluble in *water*.

DISTILLATION RANGE – Not less than 95 per cent distils between 210° and 212°, Appendix 5.3.

WT. PER ML – At 25°, about 1.20 g, Appendix 5.19.

4-Nitrobenzoyl Chloride : *p*-Nitrobenzoyl Chloride; $NO_2.C_6H_4.COCl$ = 185.57

Contains not less than 96.0 per cent of $C_7H_4ClNO_3$, as determined by both parts of the **ASSAY** described below:

DESCRIPTION – Yellow crystals or crystalline masses, decomposing in moist air.

SOLUBILITY – Completely soluble with decomposition in *sodium hydroxide solution*.

MELTING RANGE – 70° to 73°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.4 g, dissolve in 20 ml of *pyridine*, add slowly 20 ml of *water*, and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.009279 g of $C_7H_4ClNO_3$.

To the neutralised solution add 25 ml of *0.1N silver nitrate* and 5 ml of *nitric acid*, filter, wash the filter with *water*, and titrate the combined filtrate and washings with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.01856 g of $C_7H_4ClNO_3$.

4-Nitrobenzyl Bromide : *p*-Nitrobenzyl Bromide; $NO_2.C_6H_4.CH_2Br = 216.03$

DESCRIPTION – Pale yellow crystals.

SOLUBILITY – Soluble in *alcohol*.

MELTING RANGE – 96° to 100°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

4-Nitrobenzyl Chloride : *p*-Nitrobenzyl Chloride; $NO_2.C_6H_4.CH_2Cl = 171.58$

Contains not less than 98.0 per cent and not more than the equivalent to 100.0 per cent of $C_7H_6ClNO_2$.

DESCRIPTION – Pale yellow crystals; odour, slight.

MELTING RANGE – 70° to 74°, Appendix 5.11.

ASSAY – Weigh accurately about 0.5 g, add an excess of *alcoholic silver nitrate solution* and boil under a reflux condenser for one hour. Filter off the precipitate, wash, dry at 105° and weigh. Each g of residue is equivalent to 1.197 g of $C_7H_6ClNO_2$.

Nitrobenzyl Chloride Solution

A 4.5 per cent w/v solution of *nitrobenzyl chloride* in *alcohol*.

Nitrogen : $N_2 = 28.01$

Nitrogen, washed and dried.

Nitrogen, Carbon Monoxide-free

Pass a slow stream of *nitrogen* over a manganese dioxide and copper oxide catalyst heated at 100° to 120°. Pass the gas through a column of *soda lime* or an appropriate molecular sieve to remove any carbon dioxide formed.

Nitrogen, Oxygen-free

Nitrogen which has been freed from oxygen by passing it through *alkaline pyrogallol solution*.

Nitromethane : $CH_3.NO_2 = 61.04$

DESCRIPTION – A colourless oily liquid with a moderately strong, somewhat disagreeable odour.

SOLUBILITY – Slightly soluble in *water*; soluble in *alcohol*; in *solvent ether* and in *dimethyl formamide*.

BOILING POINT – About 101°, Appendix 5.3.

WT. PER ML – At 20°, about 1.13 g, Appendix 5.19.

CAUTION – Nitromethane forms explosive compounds with amines and strong bases.

Noradrenaline Acid Tartrate

Of the Indian Pharmacopoeia.

s-**Octyl Alcohol** : Octan-2-ol; $CH_3.(CH_2)_5.CH(OH).CH_3 = 130.22$

DESCRIPTION – Colourless, oily liquid; odour, characteristic.

SOLUBILITY – Insoluble in *water*; soluble in *alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 177° and 180°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.425 to 1.427, Appendix 5.14.

WT. PER ML – At 20°, 0.820 to 0.822 g, Appendix 5.19.

Octoxynol : Polyethylene Glycol Mono[*p*-(1,1,3,3-tetramethylbutyl)phenyl]Ether
$C_{34}H_{62}O_{11}$ (average) = 647 (average).

DESCRIPTION – Clear, pale yellow, viscous liquid; odour, faint; taste, bitter.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *acetone*; soluble in *benzene* and in *toluene*.

REFRACTIVE INDEX – Between 1.485 and 1.493, Appendix 5.14.

pH – Between 6.0 and 8.0, determined in a 1 per cent w/v solution, Appendix 5.10.

HYDROXYL VALUE – Between 85 and 101, Appendix 3.3.17.

WATER – Not more than 0.5 per cent w/v, Appendix 3.3.25.

CLOUD POINT – Between 65° and 75°, determined by the following method:

Weigh 1.0 g into a 250-ml beaker, and add 99 ml of *water*. Dissolve by careful heating, with stirring at a constant slow speed with a small-propeller-blade stirrer. Place a

thermometer at the centre of the liquid and heat rapidly until the entire solution becomes cloudy, then raise the temperature by 10°. Remove the source of heat, continue stirring, and note the temperature at which the solution becomes sufficiently clear to permit seeing the entire thermometer bulb clearly.

(*p*-tert-Octylphenoxy)nonaethoxyethanol

$C_{34}H_{62}O_{11}$ = 646.86.

Use suitable grade, commercially available as "Tergitol Non-ionic NPX", and as "Triton N 101".

Oestradiol Benzoate

Of the Indian Pharmacopoeia.

Orange G : Disodium 1-benzeneazo-2-hydroxynaphthalene-6,8-disulphonate; $C_6H_5.N{:}N.C_{10}H_4(OH)(SO_3Na)_2$ – 452.36

DESCRIPTION – Orange to brick-red powder or dark-red crystals.

SOLUBILITY – Readily soluble in *water*, yielding an orange yellow solution; soluble in *alcohol*; insoluble in *solvent ether* and in *chloroform*.

PURITY TESTS – (1) Dissolve about 20 mg in 10 ml of *water*, add 2 ml of *hydrochloric acid* and 0.5 g of *zinc powder*; the solution is decolourised. On filtration, the colourless filtrate, on standing exposed to air; does not regain its original colour (presence of azo group).

(2) Dissolve about 20 mg in 10 ml of *water* and add 2 ml of *sodium hydroxide solution*; a yellowish-red to red colour is produced without any precipitation.

Osmic Acid : Osmium Tetraoxide; OsO_4 = 254.20

DESCRIPTION – White or cream-coloured, very deliquescent, crystalline powder.

SOLUBILITY – Soluble in *alcohol*, in *solvent ether* and in *water*.

SULPHATED ASH – Not more than 0.025 per cent, Appendix 3.2.7.

OSMIC ACID SOLUTION – A 1.0 per cent w/v solution of *osmic acid* in *water*.

CAUTION – Osmic Acid Solution is corrosive to the eyes, mucous membranes and skin.

Oxalic Acid : $(CO_2H)_2,2H_2O$ = 126.07

Contains not less than 99.5 per cent of $C_2H_2O_4,2H_2O$, as determined by both parts of the **ASSAY**.

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

CHLORIDE – To 1 g dissolved in 20 ml of *water* add 5 ml of *dilute nitric acid* and 1 drop of *silver nitrate solution*; no turbidity is produced.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – (A) Weigh accurately about 3 g and dissolve in 50 ml of *carbon dioxide-free water* and titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.06304 g of $C_2H_2O_4,2H_2O$.

(B) Weigh accurately about 3 g, dissolve in *water*, and add sufficient *water* to produce 250 ml. To 25 ml of this solution add 5 ml of *sulphuric acid*, previously diluted with a little *water*, and titrate at a temperature of about 70° with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.006303 g of $C_2H_2O_4,2H_2O$.

Oxalic Acid and Sulphuric Acid Solution

A 5.0 per cent w/v solution of *oxalic acid* in a cooled mixture of equal volumes of *sulphuric acid* and *water*.

Oxygen

Of the Indian Pharmacopoeia.

Palladium Chloride : Palladous Chloride; $PdCl_2$ = 177.31

Contains not less than 59.3 per cent of Pd.

DESCRIPTION – Brownish-red powder; hygroscopic.

SOLUBILITY – Soluble in *water*, in *alcohol*, in *acetone*, and in *dilute hydrochloric acid*.

ASSAY – To 0.15 g add 20 ml of *5N hydrochloric acid* and warm to dissolve. Dilute to 400 ml with *water*, and add *dimethylglyoxime solution* slowly and with constant stirring until precipitation is complete. Allow to stand for 30 minutes, filter through a sintered glass crucible (grade 3, maximum pore size 15 to 40 μm), wash with cold *water*, and dry to constant weight at 105°. Each g of residue is equivalent to 0.3161 g of Pd.

Para-aminobenzoic Acid : *p*-Aminobenzoic Acid $H_2NC_6H_4.COOH$ = 137.13

DESCRIPTION – White or slightly yellow, crystals or crystalline powder, becoming discoloured on exposure to air or light; odourless.

SOLUBILITY – Freely soluble in hot *water* and in *alcohol*; soluble in *water* and in *solvent ether*; freely soluble in solutions of alkali hydroxides and carbonates; sparingly soluble in *dilute hydrochloric acid*; slightly soluble in *chloroform*.

MELTING RANGE – Between 186° and 189°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 0.2 per cent, determined by drying in an oven at 105° for two hours, Appendix 5.8.

ASSAY – Not less than 98.5 per cent, determined by the following method:

Weigh accurately about 0.3 g, previously dried at 105° for two hours, add 5 ml of *hydrochloric acid* and 50 ml of *water* and stir until dissolved. Cool to about 15° and carry out the *nitrite titration*, Appendix 3.3.4. Each ml of *0.1 M sodium nitrite* is equivalent to 0.1371 g of $C_7H_7NO_2$.

Paracetamol

Of the Indian Pharmacopoeia.

Paracetamol, 4-Aminophenol-free

Recrystallise *paracetamol* from *water* until it complies with the following test:

Dissolve 5 g of the dried material in sufficient of a mixture of equal volumes of *methyl alcohol* and *water* to produce 100 ml, add 1.0 ml of *alkaline sodium nitroprusside solution*, mix, and allow to stand for thirty minutes; no blue or green colour is produced.

Paraffin, Liquid

Of the Indian Pharmacopoeia.

Penicillinase Solution

Dissolve 10 g of *casein hydrolysate*, 2.72 g of *potassium dihydrogen phosphate* and 5.88 g of *sodium citrate* in 200 ml of *water*, adjust the alkalinity to pH 7.2 with *sodium hydroxide solution* and dilute to 1000 ml with *water*. Dissolve 0.41 g of *magnesium sulphate* in 5 ml of *water* and add 1 ml of a 0.16 per cent w/v solution of *ferrous ammonium sulphate* and sufficient *water* to produce 10 ml. Sterilise both solutions by heating in an autoclave, cool, mix, distribute in shallow layers in conical flasks, and inoculate with *Bacillus cereus* (NCTC 9946). Allow the flasks to stand at 18° to 37° until growth is apparent and then maintain at 35° to 37° C for sixteen hours, agitating constantly to ensure maximum aeration. Centrifuge and sterilise the supernatant fluid by filtration through a suitable membrane filter.

1.0 ml of Penicillinase Solution will distroy penicillin activity at the rate of at least one million Units per hour, at 30° and pH 7.0. Proportionately more is needed to destroy the activity in concentrations of 1000 Units per ml or less. To ensure complete destruction, it is advisable to use ten times the quantity of penicillinase solution indicated above.

STERILITY – Complies with the *tests for sterility*, Appendix 4.6.

Penicillinase Solution should be kept between 0° and 2° and used within two or three days. When dried from the frozen state and kept in sealed ampoules, it may be stored for several months.

Pentane : *n*-Pentane; $CH_3.(CH_2)_3.CH_3$ = 72.15

DESCRIPTION – Clear, colourless, highly flammable liquid.

SOLUBILITY – Very slightly soluble in *water*, miscible with *alcohol*, with *solvent ether*, and with many organic solvents.

WT. PER ML – At 20°, about 0.62 g, Appendix 5.19.

DISTILLATION RANGE – Not less than 95 per cent distils between 34° and 36°, Appendix 5.3.

Pepsin

Of the Indian Pharmacopoeia.

Perchloric Acid : $HClO_4$ = 100.46

A solution of perchloric acid in *water* containing between 70 to 72 per cent w/w of $HClO_4$.

DESCRIPTION – Clear, colourless liquid. It is very corrosive and may deflagrate on contact with oxidisable substances.

SOLUBILITY – Miscible with *water*.

CHLORIDE – 3 ml complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 3 ml diluted with *water* and neutralised with *dilute ammonia solution* complies with the *limit test for sulphates*, Appendix 3.2.8.

RESIDUE ON IGNITION – When ignited to constant weight, leaves not more than 0.01 per cent w/v of residue.

ASSAY – Weigh accurately about 4 g, dilute with *water* to 50 ml, and titrate with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1005 g of $HClO_4$.

Perchloric Acid, *x*M

Solutions of any molarity, *x* M may be prepared by diluting 82 *x* ml of *perchloric acid* with *water* to 1000 ml.

Perchloric Acid (60 per cent)

A solution of perchloric acid in water containing between 60.0 and 62.0 per cent w/w of $HClO_4$.

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Miscible with *water*.

CHLORIDES – 3.5 ml complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 3.5 ml diluted with *water* and neutralised

with *dilute ammonia solution* complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Carry out the **ASSAY** described under Perchloric Acid.

Perchloric Acid Solution

A 3 per cent v/v solution of *perchloric acid* in *water*.

Periodic-Acetic Acid Solution

Dissolve 0.446 g of *sodium periodate* in 2.5 ml of 25 per cent v/v *sulphuric acid* and dilute with *glacial acetic acid* to 100 ml.

Petroleum, Light : Petroleum Spirit

DESCRIPTION – Colourless, very volatile, highly flammable liquids obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons, and complying with one or other of the following definitions:

Light Petroleum (Boiling range, 30° to 40°)

WT. PER ML – At 20°, 0.620 to 0.630 g, Appendix 5.19.

Light Petroleum (Boiling range, 40° to 60°)

WT. PER ML – At 20°, 0.630 to 0.650 g, Appendix 5.19.

Light Petroleum (Boiling range, 60° to 80°)

WT. PER ML – At 20°, 0.670 to 0.690 g, Appendix 5.19.

Light Petroleum (Boiling range, 80° to 100°)

WT. PER ML – At 20°, 0.700 to 0.720 g, Appendix 5.19.

Light Petroleum (Boiling range, 100° to 120°)

WT. PER ML – At 20°, 0.720 to 0.740 g, Appendix 5.19.

Light Petroleum (Boiling range, 120° to 160°)

WT. PER ML – At 20°, about 0.75 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried at 105°, leaves not more than 0.002 per cent w/v of residue.

1,10-Phenanthroline : *o*-Phenanthroline; $C_{12}H_8N_2,H_2O$ = 198.22

DESCRIPTION – White to cream-coloured crystals, or a crystalline powder.

SOLUBILITY – Slightly soluble in *water*; freely soluble in *alcohol*.

MELTING RANGE – 97° to 102°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

SENSITIVITY – Add 0.5 ml of a 0.5 per cent w/v solution in *water* to 0.05 ml of *ferrous sulphate solution*. Dilute with a cooled mixture of 50 ml of *water* and 10 ml of *sulphuric acid*; the solution is coloured red. Add 0.1 ml of *0.1N potassium dichromate*; the colour is discharged.

Phenol : Carbolic Acid

Of the Indian Pharmacopoeia.

Phenol, Liquefied : Liquefied Carbolic Acid.

Contains 80.0 per cent w/w of Phenol (limits, 77.0 to 81.5 per cent w/w of C_6H_6O).

Phenol	800 g.
Water	Sufficient to produce 1000 g

Warm the phenol on a water-bath until it is melted, add *water*, and mix thoroughly.

DESCRIPTION – Colourless liquid which may acquire a pinkish blue on keeping; odour, characteristic and somewhat aromatic; caustic.

SOLUBILITY – Forms a clear solution on the addition of 15 parts of *water*. Miscible with *alcohol*, with *solvent ether*, and with *glycerin*.

WT. PER ML – 1.050 to 1.055 g, Appendix 5.19.

CLARITY AND ACIDITY OF SOLUTION; NON-VOLATILE MATTER – Complies with the tests stated under Phenol.

STORAGE – Preserve Liquefied Phenol in a well-closed container, protected from light. It may congeal or deposit crystals if stored below 4° but in such cases it should be completely melted before use.

NOTE – When Phenol is to be mixed with collodion, fixed oils or paraffins, melted Phenol should be used, and not Liquefied Phenol.

Phenol Solution

A saturated solution of *phenol* in *water*.

Phenoldisulphonic Acid Solution

Heat 3 g of *phenol* with 20 ml of *sulphuric acid* on a water-bath for six hours, transfer the resulting liquid to a stoppered-vessel.

DESCRIPTION – A clear liquid, which may develop a pale brown colour on storage.

SENSITIVITY TO NITRATE – Evaporate a solution containing 0.1 mg of *potassium nitrate* to dryness in a porcelain dish on a water-bath. To the cooled residue add 1 ml of the phenoldisulphonic acid solution and allow to stand for ten minutes. Add 10 ml of *water*, cool, add 10 ml of *dilute ammonia solution* and dilute to 25 ml with *water*; a distinct yellow colour is apparent when compared with a solution prepared in a similar manner but omitting the potassium nitrate.

Phenoxyacetic Acid : Phenoxyethanoic Acid

$C_6H_5.OCH_2.CO_2H = 152.14$

SOLUBILITY – Sparingly soluble in *water*; freely soluble in *alcohol*, in *solvent ether*, in *benzene*, in *carbon disulphide* and in *glacial acetic acid*.

MELTING POINT – About 99°, Appendix 5.11.

Phenoxyethanol : 2-Phenoxyethanol
$C_6H_5O.CH_2.CH_2OH = 138.17$.

DESCRIPTION – Clear, colourless, slightly viscous liquid.

SOLUBILITY – Soluble in *water*; miscible with *alcohol*, with *acetone* and with *glycerin*.

CONGEALING TEMPERATURE – Not lower than 12°, Appendix 5.5.

REFRACTIVE INDEX – At 20°, 1.536 to 1.538, Appendix 5.14.

WT. PER ML – About 1.10 g, Appendix 5.19.

***p*-Phenylenediamine Dihydrochloride :** 4-Phenylenediammonium Dichloride; $C_6H_4(NH_2)_2,2HCl = 181.06$

DESCRIPTION – White to pale tan, crystals or crystalline powder; turning red on exposure to air.

SOLUBILITY – Freely soluble in *water*; slightly soluble in *alcohol*, and in *solvent ether*.

INSOLUBLE MATTER – Dissolve 1 g in 10 ml of *water*; the solution is clear and complete.

STORAGE – Preserve *p*-Phenylenediamine Dihydrochloride in well-closed containers, protected from light.

2-Phenylethanol : Phenethyl Alcohol; $C_6H_5CH_2.CH_2OH = 122.16$

DESCRIPTION – Colourless liquid; odour, floral.

SOLUBILITY – 2 ml dissolve in 100 ml of *water* after thorough shaking; one part is clearly soluble in 1 part of *alcohol (50 per cent)*. Miscible with *alcohol*, and with *solvent ether*.

BOILING RANGE – Between 219° to 221°, Appendix 5.3.

SPECIFIC GRAVITY – Between 1.017 to 1.019, Appendix 5.19.

REFRACTIVE INDEX – At 20°, between 1.530 to 1.533, Appendix 5.14.

Phenylhydrazine : $C_6H_5.NH.NH_2 = 108.14$

DESCRIPTION – Colourless or yellowish liquid.

SOLUBILITY – Slightly soluble in *water*; soluble in *alcohol*, and in *solvent ether*.

BOILING POINT – About 241°, with some decomposition, Appendix 5.3.

CONGEALING TEMPERATURE – Not below 16°, Appendix 5.5.

STORAGE – Phenylhydrazine should be stored in a well-stoppered bottle, protected from light. It should be freshly distilled under reduced pressure before use.

Phenylhydrazine Hydrochloride : $C_6H_5.NH.NH_2,HCl = 144.60$

Contains not less than 98.0 per cent of $C_6H_8N_2,HCl$.

DESCRIPTION – White or slightly discoloured crystalline powder which becomes brown on keeping, particularly if exposed to bright light.

SOLUBILITY – Soluble in *water*, and in *alcohol*.

SULPHATED ASH – Not more than 0.5 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.2 g, and dissolve in 50 ml of *water* in a glass-stoppered flask, add 50 ml of *hydrochloric acid*, and titrate with *0.05 M potassium iodate* until the colour changes from dark to light brown, then add 5 ml of *chloroform*, and continue the titration with vigorous shaking, until the chloroform is colourless; each ml of *0.05 M potassium iodate* is equivalent to 0.007230 g of $C_6H_8N_2,HCl$.

Phenylhydrazine Solution

Dissolve 65 mg of *phenylhydrazine hydrochloride*, previously recrystallised from aqueous alcohol, in sufficient of a mixture of 170 ml of *sulphuric acid* and 80 ml of *water* to produce 100 ml.

Phloroglucinol : 1,3,5-Trihydroxybenzene;
$C_6H_3(OH)_3,2H_2O = 162.14$

DESCRIPTION – White or yellowish crystals or a crystalline powder.

SOLUBILITY – Slightly soluble in *water*; soluble in *alcohol*, and in *solvent ether*.

MELTING RANGE – After drying at 110° for one hour, 215° to 219°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

STORAGE – Phloroglucinol should be kept protected from light.

Phosphomolybdic Acid : $H_3PO_4,12MoO_3,24H_2O = 2257.65$

DESCRIPTION – Deep yellow crystals.

SOLUBILITY – Very soluble in *water*.

CLARITY OF SOLUTION – A 10 per cent w/v solution in *water* is clear.

AMMONIA – Boil 0.5 g with 20 ml of a 5 per cent w/v solution of *sodium hydroxide*; the vapour evolved does not change the colour of moistened *red litmus paper*.

Phosphomolybdic Acid Solution

A 5.0 per cent w/v solution of *phosphomolybdic acid* in *ethyl alcohol*.

Phosphoric Acid

Of the Indian Pharmacopoeia.

Phosphoric Acid, *x*N

Solutions of any normality, *x*N may be prepared by diluting 49 *x* g of *phosphoric acid* with *water* to 1000 ml.

Phosphoric Acid, Dilute

Contains approximately 10 per cent w/v of H_3PO_4.

Dilute 69 ml of *phosphoric acid* to 1000 ml with *water*.

Phosphorus Pentachloride : PCl_5 = 208.27

Contains not less than 99.0 per cent of PCl_5, as determined by both parts of the **ASSAY** described below:

DESCRIPTION – Fuming yellow solid.

ASSAY – Weigh accurately about 0.15 g and add to 50 ml of *0.1N sodium hydroxide*, and titrate with *0.1N sulphuric acid*, using *bromocresol green solution* as indicator to the green colour indicative of pH 4.5. Each ml of *0.1N sodium hydroxide* is equivalent to 0.003471 g of PCl_5.

To the neutralised liquid add 50 ml of *0.1N silver nitrate* and 5 ml of *nitric acid* and titrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.004165 g of PCl_5.

Phosphorus Pentoxide : P_2O_5 = 142.96

DESCRIPTION – White, amorphous, very deliquescent powder.

SOLUBILITY : Soluble in *water* with evolution of heat to form phosphoric acid (In making solutions, the oxide should be added to the *water* in small portions to avoid excessive sputtering).

REDUCING SUBSTANCES – Dissolve 1 g in 50 ml of *water*, add 0.5 ml of *0.1N potassium permanganate* and heat for five minutes on a water-bath; the pink colour is not completely discharged.

Phosphotungstic Acid : Approximately $24WO_3,P_2O_5,51H_2O$ = 6625.08

DESCRIPTION – White or yellowish-green crystals or a crystalline powder.

SOLUBILITY – Soluble in *water*, in *alcohol*, and in *solvent ether*.

INSOLUBLE MATTER – Dissolve 5 g in 100 ml of *water*, and warm on a water-bath for one hour. Filter the hot solution through a tared, sintered glass crucible of fine porosity. Wash the beaker and the filter thoroughly with hot *water*, dry at 105°, cool in a desiccator, and weigh. The weight of the residue should not be more than 1 mg.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

NITRATE – Dissolve 0.5 g in 10 ml of *water*, and add about 10 mg of *sodium chloride*, 0.1 ml of *indigo carmine solution* and 10 ml of *sulphuric acid*. The blue colour should not disappear within one minute.

SULPHATE – 3 g complies with the *limit test for sulphates*, Appendix 3.2.8.

Phosphotungstic Acid Solution

A 1.0 per cent w/v solution of *phosphotungstic acid* in *water*.

Phthalic Anhydride : $C_8H_4O_3$ = 148.11

DESCRIPTION – White lustrous needles.

SOLUBILITY – Slightly soluble in *water*, in *carbon disulphide*, and in *solvent ether*; soluble in *alcohol*.

MELTING POINT – About 131°, Appendix 5.11.

Picric Acid : 2,4,6-Trinitrophenol

Contains not less than 99.0 per cent of $C_6H_3N_3O_7$.

DESCRIPTION – Bright yellow, crystalline powder; odourless; taste, very bitter. Explodes when heated rapidly or subjected to percussion.

SOLUBILITY – Sparingly soluble in *water*; freely soluble in *alcohol*.

ACIDITY – A solution in *water* is intensely yellow and acid to *litmus solution*.

MELTING RANGE – 121° to 123°, Appendix 5.11 (When taking the melting point the operator should be protected by a glass screen.

SULPHATE – Dissolve 2.5 g in 50 ml of boiling *water* containing 5 ml of *dilute hydrochloric acid*; cool, and filter; 20 ml of the filtrate complies with the *limit test for sulphates*, Appendix 3.2.8.

BENZENE-INSOLUBLE MATTER – 1 g dissolves in 20 ml of *benzene* yielding a clear solution.

ASSAY – Weigh accurately about 2 g and dissolve in hot *water*. Titrate with *0.5N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.5N sodium hydroxide* is equivalent to 0.1146 g of $C_6H_3N_3O_7$.

STORAGE – Picric Acid, for safety, should be stored mixed with an equal weight of *water*.

Picric Acid Solution

A 1.0 per cent w/v solution of *picric acid* in hot *water*.

Picric Acid Solution, Alkaline

Mix 20 ml of *picric acid solution* with 10 ml of *dilute sodium hydroxide solution* and sufficient *water* to produce 100 ml.

Alkaline Picric Acid Solution should be freshly prepared.

Picrolonic Acid : 3-Methyl-4-nitro-1-(4-nitrophenyl)-5-pyrazolone; $C_{10}H_8N_4O_5$ = 264.20

DESCRIPTION – Yellow or brownish-yellow crystalline powder.

SOLUBILITY – Slightly soluble in *water*; soluble in *alcohol* and in *sodium hydroxide solution*.

MELTING RANGE – 116° to 117°, with decomposition at 125°, Appendix 5.11.

SENSITIVITY – Dissolve 25 mg in 10 ml of warm *water* containing 0.1 ml of *glacial acetic acid*; to 1 ml of this solution add 1 ml of a 0.08 per cent w/v solution of *calcium chloride* previously heated at 60°; a bulky precipitate forms within five minutes.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Piperazine Hydrate

Of the Indian Pharmacopoeia.

Platinic Chloride : Chloroplatinic Acid; $H_2PtCl_6,6H_2O$ = 517.91 or $H_2PtCl_6,3H_2O$ = 463.82

DESCRIPTION – Brownish, deliquescent, crystalline masses

SOLUBILITY – Very soluble in *water*, and in *alcohol*.

CLARITY AND COLOUR OF SOLUTION – 0.10 g dissolves completely in 1 ml of *water*, and in 1 ml of *alcohol* to yield clear solutions; the solution in *water* is yellow but not red or brown.

NITRATE – To 2 ml of a 10 per cent w/v solution in *water* add 2 ml of a 10 per cent w/v solution of *ferrous sulphate* and introduce carefully 2 ml of *sulphuric acid* so as to form a lower layer; no brownish-red colour is produced at the zone of contact in five minutes.

SULPHATE – Dilute 5 ml of a 10 per cent w/v solution with 5 ml of *water* and add 1 ml of *barium chloride solution*; no turbidity or precipitate develops within one hour.

Platinic Chloride Solution

A solution of *platinic chloride* in *water*, containing the equivalent of 5.0 per cent w/v of $H_2PtCl_6,6H_2O$.

Polyethylene Glycol 400 : Macrogel 400

DESCRIPTION – Clear, colourless or almost colourless, viscous liquid.

SOLUBILITY – Miscible with *water*; very soluble in *alcohol*, in *chloroform* and in *acetone*; practically insoluble in *solvent ether*, and in fixed oils.

WT. PER ML – At 20°, about 1.12 g, Appendix 5.19.

VISCOSITY – At 100°, about 7.1 centistokes, Appendix 5.18.

Polyethylene Glycol 600 : Macrogol 600

DESCRIPTION – Clear, practically colourless, viscous liquid, having a slight characteristic odour. Slightly hygroscopic.

SOLUBILITY – Soluble in *water*, in *acetone*, in *alcohol*, in other glycols and in aromatic hydrocarbons; insoluble in *solvent ether* and in aliphatic hydrocarbons.

VISCOSITY – At 100°, between 9.9 centistokes and 11.1 centistokes, Appendix 5.18.

Polyethylene Glycol 1000 : Macrogel 1000

DESCRIPTION – White solid mass with a waxy appearance; odourless.

SOLUBILITY – Soluble in *water*; freely soluble in *alcohol* and in *chloroform*; practically insoluble in *solvent ether*, and in fixed oils.

VISCOSITY – At 100°, about 17.3 centistokes, Appendix 5.18.

Polyethylene Glycol 1500 : Macrogel 1500

Of the Indian Pharmacopoeia.

Polysorbate 20

Of the Indian Pharmacopoeia.

Polysorbate 80

Of the Indian Pharmacopoeia.

Potassium Acetate : CH_3COOK = 98.14

Contains not less than 99.0 per cent of $C_2H_3O_2K$, calcula-

ted with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White powder, or granules or white crystals; odourless or having a faint acetous odour; taste, saline and slightly alkaline; very deliquescent.

SOLUBILITY – Very soluble in *water*; freely soluble in *alcohol*.

ALKALINITY – 1 g dissolved in 20 ml of recently boiled and cooled *water*, requires not more than 0.5 ml of *0.1N sulphuric acid* to produce a solution which is acid to *phenolphthalein solution*.

ALUMINIUM AND CALCIUM – Dissolve 0.5 g in 25 ml of *water* and warm with 1 ml of *dilute ammonia solution* and 1 ml of *ammonium oxalate solution*; no precipitate or turbidity is produced.

SODIUM – Dissolve 0.3 g in 4.5 ml of *water*, add 1 ml of *alcohol* and 3 ml of *potassium antimonate solution*, and allow to stand; no sediment or white, crystalline precipitate is visible to the naked eye within fifteen minutes.

CHLORIDE – 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

LOSS ON DRYING – Loses not more than 5.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

ASSAY – Weigh accurately about 2 g, heat until carbonised, cool, add 50 ml of *water* and 50 ml of *0.5N sulphuric acid* and boil; filter, wash the filter with *water*, combine the filtrate and washings, and titrate the excess of the acid with *0.5N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *0.5N sulphuric acid* is equivalent to 0.04907 g of $C_2H_3O_2K$.

Potassium Antimonate : $KSbO_3,3H_2O$ = 262.90

Contains not less than 40.0 per cent of Sb.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Sparingly soluble in *water*, very slowly soluble in the cold, but rapidly soluble on boiling.

ASSAY – Weigh accurately about 0.3 g, and dissolve in 100 ml of *water*, add 2 ml of *dilute hydrochloric acid*, and pass in *hydrogen sulphide* until the antimony is completely precipitated. Add 2 ml of *hydrochloric acid* and again pass in *hydrogen sulphide*. Boil, filter, wash the precipitate with hot *water* saturated with *hydrogen sulphide*, and dissolve the precipitate in 25 ml of *hydrochloric acid*. Boil to remove hydrogen sulphide, and dilute to 50 ml with *water*. Add 2 g of *sodium potassium tartrate*, neutralise carefully with *sodium carbonate*, add 2 g *sodium bicarbonate*, and titrate with *0.1N iodine*, using *starch solution* as indicator. Each ml of *0.1N iodine* is equivalent to 0.006088 g of Sb.

Potassium Antimonate Solution

Boil 2 g of *potassium antimonate* with 95 ml of *water* until dissolved. Cool rapidly and add 50 ml of *potassium hydroxide solution* and 5 ml of *N sodium hydroxide*. Allow to stand twenty-four hours, filter and add sufficient *water* to produce 150 ml.

SENSITIVITY TO SODIUM – To 10 ml add 7 ml of *0.1M sodium chloride*; a white, crystalline precipitate is formed within fifteen minutes.

Potassium Antimonate Solution should be freshly prepared.

Potassium Bicarbonate : Potassium Hydrogen Carbonate; $KHCO_3$ = 100.12

Contains not less than 99.0 per cent of $KHCO_3$.

DESCRIPTION – Colourless, transparent, monoclinic prisms, or a white, granular powder.

SOLUBILITY – Freely soluble in *water*; practically insoluble in *alcohol*.

ALKALINITY – pH of a 1.0 per cent w/v solution, not greater than 8.6, Appendix 5.10.

ALUMINIUM, CALCIUM, AND INSOLUBLE MATTER – Boil 5.0 g with 50 ml of *water* and 10 ml of *dilute ammonia solution*, filter, and wash; the insoluble residue, after ignition, weighs not more than 5 mg.

COLOUR AND CLARITY OF SOLUTION – 5 g dissolved in 50 ml of *water* is clear and colourless.

ASSAY – Dissolve 1.5 g in 20 ml of *water* and titrate with *0.5N hydrochloric acid*, using *bromophenol blue solution* as indicator. Each ml of *0.5N hydrochloric acid* is equivalent to 0.05006 g of $KHCO_3$.

Potassium Bisulphate : Potassium Hydrogen Sulph
$KHSO_4$ = 136.16

Contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $KHSO_4$.

DESCRIPTION – Fused, white lumps; hygroscopic.

SOLUBILITY – Very soluble in *water*, giving an acid solution.

IRON – 2 g complies with the *limit test for iron*, Appendix 3.2.5.

ASSAY – Weigh accurately about 4.5 g, dissolve in 50 ml of *water* and titrate with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1362 g of $KHSO_4$.

Potassium Bromate : $KBrO_3$ = 167.00

Contains not less than 99.8 per cent of $KBrO_3$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*, freely soluble in boiling *water*; almost insoluble in *alcohol*.

ACIDITY OR ALKALINITY – A 5 per cent w/v solution in *water* is clear and colourless and neutral to *litmus solution*.

SODIUM – A warm 10 per cent w/v solution in *water*, tested on platinum wire, imparts no distinct yellow colour to a colourless flame.

BROMIDE – To 20 ml of a 5 per cent w/v solution in *water*, add 1 ml of *0.1N sulphuric acid*; no yellow colour develops within one minute, comparison being made with a control solution to which no acid has been added.

SULPHATE – 1 g complies with the *limit test for sulphates*, Appendix 3.2.8.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 0.1 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 1 g, dissolve in *water* and dilute to 250 ml. To 25 ml of this solution add 3 g of *potassium iodide* and 10 ml of *hydrochloric acid*, dilute with 100 m of water and titrate with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.002783 g of $KBrO_3$.

Potassium Bromide

Of the Indian Pharmacopoeia.

Potassium Bromide, 0.001 N

Dissolve 0.1190 g of *potassium bromide* in sufficient *water* to produce 1000 ml.

Potassium Bromide, IR

Use spectroscopic reagent grade complying with the following test:

The *infra-red absorption spectrum*, Appendix 5.15 B, of a disc prepared from material previously dried at 250° for one hour, has a substantially flat base line over the range 3800 to 625 cm^{-1}; it exhibits no maxima with an extinction greater than 0.1 above the base line with the exception of maxima due to water at 3440 and 1630 cm^{-1}

Potassium Carbonate : K_2CO_3 = 138.21

Contains not less than 98.0 per cent of K_2CO_3.

DESCRIPTION – White, granular powder; hygroscopic.

SOLUBILITY – Very soluble in *water*, forming a clear solution.

IRON – 1 g, with the addition of 1.5 ml of *hydrochloric acid*, complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – 1 g, with the addition of 5 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 1 g, with the addition of 5 ml of *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

CHROMIUM – To 25 ml of a 2 per cent w/v solution in *water*, add about 0.2 g of *sodium peroxide* and boil gently for five minutes, cool, acidify with *dilute sulphuric acid* and add 2 drops of *diphenylcarbazide solution*; no violet colour is produced.

ASSAY – Weigh accurately about 3 g, dissolve in 50 ml of *water*, and titrate with *N hydrochloric acid*, using *bromophenol blue solution* as indicator. At the first colour change, boil the solution, cool, and complete the titration. Each ml of *N hydrochloric acid* is equivalent to 0.06911 g of K_2CO_3.

Potassium Carbonate, Anhydrous

Potassium Carbonate dried at 135° for two hours spread in a thin layer and then cooled in a desiccator.

Potassium Chlorate : $KClO_3$ = 122.55

Contains not less than 99.0 per cent of $KClO_3$.

DESCRIPTION – White powder or colourless crystals. In admixture with organic or readily oxidisable substances, it is liable to explode if heated or subjected to percussion or trituration.

SOLUBILITY – Soluble in *water*, and in *glycerin*; practically insoluble in *alcohol*.

LEAD – Not more than 10 parts per million, Appendix 3.2.6.

CHLORIDE – 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 0.3 g and dissolve in 10 ml of *water* in a stoppered flask, add 1 g of *sodium nitrite* dissolved in 10 ml of *water* and then 20 ml of *nitric acid*; stopper the flask and allow to stand for ten minutes; add 100 ml of *water* and sufficient *potassium permanganate solution* to produce a permanent pink colour; decolorise by the addition of a trace of *ferrous sulphate* and add 0.1 g of *urea*. Add 30 ml of *0.1N silver nitrate*, filter, wash with *water*, and titrate the filtrate and washings with

0.1N ammonium thiocyanate, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.01226 g of $KClO_3$.

Potassium Chloride

Of the Indian Pharmacopoeia.

Potassium Chloride IR

Use spectroscopic reagent grade complying with the following test:

The *infra-red absorption spectrum*, Appendix 5.15 B, of a disc prepared from material previously dried at 250° for one hour, has a substantially flat base line over the range 3800 to 625 cm^{-1}; it exhibits no maxima with an extinction greater than 0.1 above the base line with the exception of maxima due to water at 3440 and 1630^{-1}:

Potassium Chromate : K_2CrO_4 = 194.19

Contains not less than 99.0 per cent of K_2CrO_4.

DESCRIPTION – Yellow crystalline solid.

SOLUBILITY – Very soluble in *water*, forming a clear solution.

ALKALINITY – Dissolve 1 g in 50 ml of freshly boiled and cooled *water* and add 2 drops of *phenolphthalein solution*; any pink colour produced is discharged by the addition of not more than 0.2 ml of *0.1N sulphuric acid*.

SODIUM – A 10 per cent w/v solution in *water*, tested on a platinum wire, imparts no distinct yellow colour to a colourless flame.

CHLORIDE – To 20 ml of a 5 per cent w/v solution in *water*, add 10 ml of *nitric acid*, warm to about 50° and add a few drops of *silver nitrate solution*; not more than a faint opalescence is produced.

ASSAY – Weigh accurately about 3 g, dissolve in freshly boiled and cooled *water* and dilute to 250 ml. Transfer 25 ml of this solution to a glass-stoppered flask, add 2 g of *potassium iodide* and 10 ml of *hydrochloric acid*; and allow to stand in the dark for ten minutes. Add about 200 ml of freshly boiled and cooled *water* and titrate with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.006474 g of K_2CrO_4.

Potassium Chromate Solution

A 5.0 per cent w/v solution of *potassium chromate* in *water*.

Potassium Citrate

Of the Indian Pharmacopoeia.

Potassium Cupri-tartrate Solution : Cupric Tartrate Alkaline Solution; Fehling's Solution

(1) COPPER SOLUTION – Dissolve 34.66 g of carefully selected small crystals of *copper sulphate*, showing no trace of efflorescence or of adhering moisture, in sufficient *water* to make 500 ml. Keep this solution in small, well-stoppered bottles.

(2) ALKALINE TARTRATE SOLUTION – Dissolve 176 g of *sodium potassium tartrate* and 77 g of *sodium hydroxide* in sufficient *water* to produce 500 ml.

Mix equal volumes of the solutions No. 1 and No. 2 at the time of using.

Potassium Cyanide : KCN = 65.12

Contains not less than 95.0 per cent of KCN.

DESCRIPTION – White, crystalline powder, gradually decomposing on exposure to air.

SOLUBILITY – Readily soluble in *water*, forming a clear, colourless solution.

HEAVY METALS – To 20 ml of a 5 per cent w/v solution in *water*, add 10 ml of *hydrogen sulphide solution*; no darkening is produced immediately or on the addition of 5 ml of *dilute hydrochloric acid*.

ASSAY – Weigh accurately about 0.5 g and dissolve in 50 ml of *water*, add 5 ml of *dilute ammonia solution* and 1 drop of *potassium iodide solution*; titrate with *0.1N silver nitrate* until a faint permanent turbidity appears. Each ml of *0.1N silver nitrate* is equivalent to 0.01302 g of KCN.

Potassium Cyanide Solution

A 10.0 per cent w/v solution of *potassium cyanide* in *water*.

Potassium Cyanide Solution, Lead-free

Weigh accurately about 10 g of *potassium cyanide* and dissolve in 90 ml of *water*, add 2 ml of *hydrogen peroxide solution*, allow to stand for twenty-four hours, and make up to 100 ml with *water*. It complies with the following test:

Mix 2 ml with 5 ml of *lead-free ammonia solution* and 40 ml of *water*, and add 5 ml of *standard lead solution*; no darkening is produced.

Potassium Dichromate : $K_2Cr_2O_7$ = 294.18

Contains not less than 99.8 per cent of $K_2Cr_2O_7$.

DESCRIPTION – Orange-red crystals or a crystalline powder.

SOLUBILITY – Soluble in *water*.

CHLORIDE – To 20 ml of a 5 per cent w/v solution in *water* add 10 ml of *nitric acid*, warm to about 50° and add a few

drops of *silver nitrate solution*; not more than a faint opalescence is produced.

ASSAY – Carry out the **ASSAY** described under Potassium Chromate, using 2 g. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.004904 g of $K_2Cr_2O_7$.

Potassium Dichromate Solution

A 7.0 per cent w/v solution of *potassium dichromate* in *water*.

Potassium Dichromate Solution UV

Dry a quantity of *potassium dichromate* by heating to constant weight at 130°. Weigh accurately a quantity not less than 57.0 mg and not more than 63.0 mg and dissolve in sufficient *0.01N sulphuric acid* to produce 1000 ml.

Potassium Dihydrogen Citrate : Potassium Citrate Monobasic; $KH_2C_6H_5O_7 = 230.21$

DESCRIPTION – White crystalline powder.

SOLUBILITY – Soluble in *water*.

Potassium dihydrogen phosphate : $KH_2PO_4 = 136.09$

Contains not less than 99.5 per cent of KH_2PO_4.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Readily soluble in *water*, forming a clear colourless solution.

ACIDITY – A 1 per cent w/v solution in *carbon dioxide-free water* gives, with *bromocresol green solution*, the green colour indicative of pH 4.4.

IRON – To a 5.0 per cent w/v solution in water, which is colourless, add *dilute ammonia solution* until the solution is alkaline to *litmus paper*, the solution remains clear and colourless.

LOSS ON DRYING – When dried at 105° for one hour, loses not more than 0.1 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 5 g, dissolve in 100 ml of *water* and titrate with *N sodium hydroxide*, using *thymol blue solution* as indicator and titrating to the colour indicative of pH 9.2. Each ml of *N sodium hydroxide* is equivalent to 0.1361 g of KH_2PO_4.

Potassium Dihydrogen Phosphate, M

Dissolve 136.09 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000 ml.

Potassium Dihydrogen Phosphate Solution

Dissolve 1.43 g of *potassium dihydrogen phosphate* in sufficient *water* to produce 1000.0 ml and dilute 10.0 ml to 1000.0 ml with *water* immediately before use. It contains 0.01 mg of phosphate (PO_4) per ml.

Potassium Ferricyanide : $K_3Fe(CN)_6 = 329.25$

Contains not less than 99.0 per cent of $K_3Fe(CN)_6$.

DESCRIPTION – Ruby-red crystals.

SOLUBILITY – Very soluble in *water*.

FERROCYANIDE – Rapidly wash 1 g with *water*, then dissolve in 100 ml of *water* and add 1 drop of *ferric ammonium sulphate solution*; no blue colour is produced.

ASSAY – Weigh accurately about 1 g and dissolve in 50 ml of *water*, add 5 g of *potassium iodide* and 3 g of *zinc sulphate*, and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.03293 g of $K_3Fe(CN)_6$.

Potassium Ferricyanide Solution

Wash about 1 g of potassium ferricyanide crystals with a little *water*, and dissolve the washed crystals in 100 ml of *water*.

Potassium Ferricyanide solution must be freshly prepared.

Potassium Ferrocyanide : $K_4Fe(CN)_6,3H_2O = 422.39$

Contains not less than 99.0 per cent of $K_4Fe(CN)_6,3H_2O$.

DESCRIPTION – Yellow, crystalline powder.

SOLUBILITY – Soluble in *water*.

ACIDITY OR ALKALINITY – A 10 per cent w/v solution in *water* is neutral to *litmus paper*.

ASSAY – Weigh accurately about 1 g and dissolve in 200 ml of *water*, add 10 ml of *sulphuric acid* and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.04224 g of $K_4Fe(CN)_6,3H_2O$.

Potassium Ferrocyanide Solution

A 5.0 per cent w/v solution of *potassium ferrocyanide* in *water*.

Potassium Hydrogen Phthalate : $CO_2H.C_6H_4.CO_2K = 204.22$

Contains not less than 99.9 per cent and not more than the equivalent of 100.1 per cent of $C_8H_5O_4K$, calculated with reference to the substance dried at 110° for one hour.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Slowly soluble in *water*, forming clear, colourless solution.

ACIDITY : A 2.0 per cent w/v solution in *carbon dioxide-free water* gives with *bromophenol blue solution* the grey colour indicative of pH 4.0.

LOSS ON DRYING – When dried at 105° for one hour, loses not more than 0.1 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 9 g, dissolve in 100 ml of *water* and titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.2042 g of $C_8H_5O_4K$.

Potassium Hydrogen Phthalate, 0.02 M

Dissolve 4.084 g of *potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydrogen Phthalate, 0.2 M

Dissolve 40.84 g of *potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydrogen Tartrate : Potassium Acid Tartrate; COOH.CH(OH).CH(OH).COOK = 188.18

Contains not less than 99.5 per cent of $C_4H_5O_6K$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – Colourless, slightly opaque crystals, or a gritty; white, crystalline powder; odourless; taste, pleasant and acid.

SOLUBILITY – Slightly soluble in *water*; sparingly soluble in boiling *water*; practically insoluble in *alcohol*.

ACIDITY – pH of a 0.5 per cent w/v solution in *carbon dioxide-free water*, 3.5 to 4.0, Appendix 5.10.

COPPER AND IRON – Dissolve 2 g in 40 ml of *water* and 5 ml of *dilute ammonia solution* and add five drops of *sodium sulphide solution*; the colour produced is not more than slightly deeper than that given by a similar solution containing in addition 1 ml of *potassium cyanide solution*.

FREE TARTARIC ACID – Shake 1 g with 20 ml of *alcohol (90 per cent)*, filter, evaporate 10 ml of the filtrate to dryness and dry at 105°; the residue weighs not more than 1 mg.

LOSS ON DRYING – Loses not more than 1.0 per cent of its weight when dried to constant weight at 105°, Appendix 5.8.

ASSAY – Weigh accurately about 1.5 g, dissolve in 100 ml of boiling *water*, and titrate, while hot, with *0.2 N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *0.2 N sodium hydroxide* is equivalent to 0.03764 g of $C_4H_5O_6K$.

Potassium Hydroxide : Caustic Potash; KOH = 56.11

Contains not less than 85.0 per cent of total alkali, calculated as KOH and not more than 4.0 per cent of K_2CO_3.

DESCRIPTION – Dry, white sticks, pellets or fused mass; hard, brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

SOLUBILITY – Freely soluble in *water*, in *alcohol*, and in *glycerin*; very soluble in boiling *ethyl alcohol*.

ALUMINIUM, IRON, AND MATTER INSOLUBLE IN HYDROCHLORIC ACID – Boil 5 g with 40 ml of *dilute hydrochloric acid*, cool, make alkaline with *dilute ammonia solution*, boil, filter, and wash the residue with a 2.5 per cent w/v solution of *ammonium nitrate*; the insoluble residue, after ignition to constant weight, weighs not more than 5 mg.

ARSENIC – Not more than 4 parts per million, Appendix 3.2.1.

CHLORIDE – 0.5 g dissolved in *water* with the addition of 1.6 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

HEAVY METALS – Dissolve 1 g in a mixture of 5 ml of *water* and 7 ml of *dilute hydrochloric acid*. Heat to boiling, add 1 drop of *phenolphthalein solution* and *dilute ammonia solution* dropwise to produce a faint pink colour. Add 2 ml of *acetic acid* and *water* to make 25 ml; the limit of heavy metals is 30 parts per million, Appendix 3.2.4.

SULPHATE – Dissolve 1 g in *water* with the addition of 4.5 ml of *hydrochloric acid*; the solution complies with the *limit test for sulphates*, Appendix 3.2.8.

SODIUM – To 3 ml of a 10 per cent w/v solution add 1 ml of *water*, 1.5 ml of alcohol, and 3 ml of *potassium antimonate solution* and allow to stand; no white crystalline precipitate or sediment is visible to the naked eye within fifteen minutes.

ASSAY – Weigh accurately about 2 g, and dissolve in 25 ml of *water*, add 5 ml of *barium chloride solution*, and titrate with *N hydrochloric acid*, using *phenolphthalein solution* as indicator. To the solution in the flask add *bromophenol blue solution*, and continue the titration with *N hydrochloric acid*. Each ml of *N hydrochloric acid*, used in the second titration is equivalent to 0.06911 g of K_2CO_3. Each ml of *N hydrochloric acid*, used in the combined titration is equivalent to 0.05611 g of total alkali, calculated as KOH.

STORAGE – Potassium Hydroxide should be kept in a well-closed container.

Potassium Hydroxide, *x*N

Solution of any normality, *x*N, may be prepared by dissolving 56.11*x* g of *potassium hydroxide* in *water* and diluting to 1000 ml.

Potassium Hydroxide Solution : Solution of Potash

An aqueous solution of *potassium hydroxide* containing 5.0 per cent w/v of total alkali, calculated as KOH (limits, 4.75 to 5.25).

ASSAY – Titrate 20 ml with *N sulphuric acid*, using solution of *methyl orange* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.05611 g of total alkali, calculated as KOH.

STORAGE – Potassium Hydroxide Solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Hydroxide Solution, Alcoholic

A 10.0 per cent w/v solution of *potassium hydroxide* in *alcohol*.

Alcoholic Potassium Hydroxide Solution must be recently prepared.

Potassium Iodate : KIO_3 = 214.00

Contains not less than 99.8 per cent of KIO_3, calculated with reference to the substance dried at 105° for one hour.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Soluble in *water*.

ACIDITY OR ALKALINITY – A 5 per cent w/v solution in *water* is neutral to *litmus paper*.

CHLORATE – To 2 g of the powdered salt add 2 ml of *sulphuric acid*; the salt remains white and no odour or gas is evolved.

IODIDE – Dissolve 1 g in 20 ml of *water*, add 1 ml of *0.1N sulphuric acid* and 2 ml of *chloroform*, and shake vigorously; no violet colour appears in the chloroform layer.

SULPHATE – Dissolve 1 g in 25 ml of *water*, add 10 ml of *hydrochloric acid*, heat nearly to boiling, add 1 ml of *barium chloride solution* and allow to stand for ten minutes; no turbidity or precipitate is produced.

LOSS ON DRYING – When dried at 105° for one hour, loses not more than 0.1 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 1.5 g, dissolve in *water* and dilute to 250 ml. To 25 ml of this solution add 3 g of *potassium iodide*, 10 ml of *hydrochloric acid* and 100 ml of *water*, and titrate with *0.1N sodium thiosulphate*, using *starch solution* as indicator, added towards the end of the titration. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003567 g of KIO_3.

Potassium Iodate Solution

A 1.0 per cent w/v solution of *potassium iodate* in *water*.

Potassium Iodide

Of the Indian Pharmacopoeia.

Potassium Iodide, M

Dissolve 166.00 g of *potassium iodide* in sufficient *water* to produce 1000 ml.

Potassium Iodide and Starch Solution

Dissolve 10 g of *potassium iodide* in sufficient *water* to produce 95 ml and add 5 ml of *starch solution*.

Potassium Iodide and Starch Solution must be recently prepared.

Potassium Iodide Solution

A 10 per cent w/v solution of *potassium iodide* in *water*.

Potassium Iodide Solution, Saturated

A saturated solution of *potassium iodide* in freshly boiled *water*; it contains some undissolved crystals.

Saturated Potassium Iodide Solution should be protected from light and should be discarded if it fails to comply with the following test:

Add 0.1 ml of *starch solution* to 0.5 ml of the solution in 30 ml of a mixture of 3 volumes of *6N acetic acid* and 2 volumes of *chloroform*; if a blue colour is formed it is discharged by the addition of not more than 0.05 ml of *0.1N sodium thiosulphate*.

Potassium Iodobismuthate Solution

Dissolve 100 g of *tartaric acid* in 400 ml of *water* and add 8.5 g of *bismuth oxynitrate*. Shake during one hour, add 200 ml of a 40 per cent w/v solution of *potassium iodide*, and shake well. Allow to stand for twenty-four hours and filter.

Potassium Iodobismuthate Solution, Dilute

Dissolve 100 g of *tartaric acid* in 500 ml of *water* and add 50 ml of *potassium iodobismuthate solution*.

Potassium Iodoplatinate Solution

Add 50 ml of a 5 per cent w/v solution of *platinic chloride* to 45 ml of *potassium iodide solution* and dilute to 100 ml with *water*.

Potassium Iodoplatinate Solution should be stored in an amber glass container.

Potassium Mercuri-iodide Solution : Mayer's Reagent

Add 1.36 g of *mercuric chloride* dissolved in 60 ml of *water* to a solution of 5 g of *potassium iodide* in 20 ml of *water*, mix and add sufficient *water* to produce 100 ml.

Potassium Mercuri-iodide Solution, Alkaline : Nessler's Reagent

To 3.5 g of *potassium iodide* add 1.25 g of *mercuric chloride* dissolved in 80 ml of water, add a cold saturated solution of *mercuric chloride* in *water*, with constant stirring

until a slight red precipitate remains. Dissolve 12 g of *sodium hydroxide* in the solution, add a little more of the cold saturated solution of *mercuric chloride* and sufficient water to produce 100 ml. Allow to stand and decant the clear liquid.

Potassium Nitrate : KNO_3 = 101.10

Contains not less than 99.0 per cent of KNO_3.

DESCRIPTION – Colourless crystals or a white crystalline powder; odourless; taste, cool and saline.

SOLUBILITY – Freely soluble in *water*.

HEAVY METALS – Dissolve 1 g in 15 ml of *water* and 2 ml of *dilute acetic acid* and add *water* to produce 25 ml; the limit of heavy metals is 10 parts per million, Appendix 3.2.4.

SODIUM – A 5 per cent w/v solution tested on a platinum wire imparts no distinct yellow colour to a colourless flame.

CHLORIDE – Dissolve 2 g in 50 ml of *water* and add 1 ml of *dilute nitric acid* and 0.8 ml of *silver nitrate solution*; no opalescence is produced.

SULPHATE – 1 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 0.3 g and dissolve in 300 ml of *water* in an ammonia distillation apparatus; add 3 g of *Devarda's alloy* and 10 ml of *sodium hydroxide solution*, and distil. Collect the distillate in 50 ml of *0.1N hydrochloric acid* and titrate the excess of acid with *0.1N sodium hydroxide*, using *methyl red solution* as indicator. Repeat the operation without the potassium nitrate. The difference between the titrations represents the acid required to neutralise the ammonia formed from the potassium nitrate. Each ml of *0.1N hydrochloric acid* is equivalent to 0.01011 g of KNO_3.

Potassium Permanganate

Of the Indian Pharmacopoeia.

Potassium Permanganate Solution

A 1.0 per cent w/v solution of *potassium permanganate* in *water*.

Potassium Permanganate Solution, Strong

A 3.0 per cent w/v solution of *potassium permanganate* in *water*.

Potassium Permanganate and Phosphoric Acid Solution

Dissolve 3 g of *potassium permanganate* in a mixture of 15 ml of *phosphoric acid* and 70 ml of *water*, add sufficient *water* to produce 100 ml.

Potassium Plumbite Solution

Dissolve 1.7 g of *lead acetate*, 3.4 g of *potassium citrate* and 50 g of *potassium hydroxide* in sufficient *water* to produce 100 ml.

Potassium Solution, FP

Dissolve 1.1440 g of *potassium chloride*, previously dried to constant weight at 130°, in sufficient freshly distilled *water* to produce 1000.0 ml.

Potassium Solution FP contains 600 µg of K per ml.

Potassium Sulphate : K_2SO_4 = 174.25

DESCRIPTION – White or colourless crystals.

SOLUBILITY – Soluble in *water*.

ACIDITY OR ALKALINITY – A 5 per cent w/v solution in *water* is neutral to *litmus paper*.

AMMONIA – Dissolve 1 g in 50 ml of *ammonia-free water* and add 1 ml of *alkaline potassium mercuri-iodide solution*; any colour produced is not deeper than that obtained by mixing 1 ml of *alkaline potassium mercuri-iodide solution* with 2 ml of *dilute ammonium chloride solution (Nessler's)* and 47 ml of *ammonia-free water*.

NITRATE – Dissolve 1 g in 10 ml of *water*, add 1 ml of *indigo carmine solution*, 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the blue colour does not entirely disappear.

LOSS ON IGNITION – When ignited to constant weight, loses not more than 1.0 per cent of its weight.

Potassium Tetraoxalate : $KH_3(C_2H_4)_2,2H_2O$ – 254.19

DESCRIPTION – White crystalline powder.

SOLUBILITY – Sparingly soluble in *water*, soluble in boiling *water*, slightly soluble in *alcohol*.

Potassium Thiocyanate : KCNS = 97.18

Contains not less than 99.0 per cent of KCNS, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – Colourless crystals; deliquescent.

SOLUBILITY – Very soluble in *water*; soluble in *ethyl alcohol*.

ALKALINITY – A 10 per cent w/v solution in *carbon dioxide-free water* is not alkaline to *bromothymol blue solution*.

AMMONIA – Boil 1 g with 5 ml of *sodium hydroxide solution*; no ammonia is evolved.

CHLORIDE – Dissolve 1 g in a solution of *ammonium nitrate* in 30 ml of *hydrogen peroxide solution* contain-

ing not more than 1 part per million of chloride, add 1 g of *sodium hydroxide*, gently warm and when the vigorous reaction subsides add a further 30 ml of the *hydrogen peroxide solution* and boil for two minutes, cool, add 5 ml of *nitric acid* and 1 ml of *silver nitrate solution*; any opalescence produced is not greater than that produced by treating 1 ml of *0.01N hydrochloric acid* in the same manner.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

OTHER SULPHUR COMPOUNDS – Dissolve 1 g in 50 ml of *water*, add 2 ml of *dilute hydrochloric acid*, and titrate with *0.1N iodine*; not more than 0.5 ml of *0.1N iodine* is required.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 2.0 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 0.4 g and dissolve in 50 ml of *water*. Add 5 ml of *nitric acid*, 50 ml of *0.1N silver nitrate*, and 5 ml of *ferric ammonium sulphate solution*, and titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*. Each ml of *0.1N silver nitrate* is equivalent to 0.009718 g of KCNS.

Potassium Thiocyanate Solution

A 9.7 per cent w/v solution of *potassium thiocyanate* in *water*.

Procaine Hydrochloride

Of the Indian Pharmacopoeia.

n-Propyl Alcohol

: Propyl Alcohol; Propan-1-ol; $CH_3.CH_2.CH_2OH$ = 60.10

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Miscible with *water*, and with *alcohol*.

DISTILLATION RANGE – Not less than 95 per cent distils between 96° and 99°, Appendix 5.3.

WT. PER ML – At 20°, 0.802 to 0.806 g, Appendix 5.19.

NON-VOLATILE MATTER – When evaporated on a water-bath and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

Propylene Glycol

Of the Indian Pharmacopoeia.

Pumice Powder

The particles of pumice of commerce, powdered, and sifted, which pass through a *No. 22 sieve*, but are retained by a *No. 60 sieve*.

Pumice Stone

A substance of volcanic origin consisting chiefly of complex silicates of aluminium and alkali metals.

DESCRIPTION – Very light, hard, rough, porous, greyish masses; odourless and tasteless; stable in air.

SOLUBILITY – Practically insoluble in *water*.

ACID- AND WATER-SOLUBLE SUBSTANCES – Boil 2 g of powdered pumice stone with 50 ml of *dilute hydrochloric acid* under reflux condenser for thirty minutes. Cool, and filter. To half of the filtrate add 5 drops of *sulphuric acid*, evaporate to dryness, ignite, and weigh. The residue weighs not more than 60 mg (6.0 per cent).

Pyridine

: C_5H_5N = 79.10

Contains not less than 99.0 per cent w/w of C_5H_5N.

DESCRIPTION – Clear, colourless liquid; odour, characteristic and unpleasant; hygroscopic.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *chloroform*.

DISTILLATION RANGE – Not more than 95.0 per cent distils between 114° and 117°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.509 to 1.510, Appendix 5.14.

WT. PER ML – At 25°, about 0.98 g, Appendix 5.19.

AMMONIA – Dissolve 2 ml in 10 ml of *carbon dioxide-free water* and add 0.1 ml of *phenolphthalein solution*; no pink colour is produced.

CHLORIDE – Dissolve 2 ml in 35 ml of *water*, add 15 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; no opalescence is produced.

READILY OXIDISABLE SUBSTANCES – Mix 10 ml with 0.1 ml of *0.1N potassium permanganate* and allow to stand for one hour. The pink colour does not entirely disappear.

WATER – Not more than 0.02 per cent w/w, Appendix 3.3.25.

WATER-INSOLUBLE MATTER – Mix 1 ml with 9 ml of *water*; the resulting solution remains clear.

NON-VOLATILE MATTER – When evaporated and dried to constant weight at 105°, leaves not more than 0.01 per cent w/v of residue.

ASSAY – Weigh accurately about 3 g, dissolve in 20 ml of *water*, and titrate with *N hydrochloric acid*, using *bromophenol blue solution* as indicator and titrating to the full yellow colour indicative of pH 2.8. Each ml of *N hydrochloric acid* is equivalent to 0.07910 g of C_5H_5N.

Pyridine, Anhydrous

Dry *pyridine* over *anhydrous sodium carbonate*. Filter and distill. Complies with the following test:

WATER – Not more than 0.01 per cent w/w, Appendix 3.3.25.

Pyridine Bromide Solution

Dissolve 8 g of *pyridine* and 5.4 ml of *sulphuric acid* in 20 ml of *glacial acetic acid*, keeping the mixture cool. Add 2.6 ml of *bromine* dissolved in 20 ml of *glacial acetic acid* and dilute with *glacial acetic acid* to 1000 ml.

Pyridine Bromide Solution should be freshly prepared.

Pyridine, Dehydrated

Use *anhydrous pyridine*.

Pyridine Hydrochloride

Of the Indian Pharmacopoeia.

Pyrogallol : 1,2,3-Trihydroxybenzene; $C_6H_3(OH)_3$ = 126.11

DESCRIPTION – White or slightly discoloured, light or heavy crystals.

SOLUBILITY – Very soluble in *water*, forming a solution which absorbs oxygen and becomes dark brown.

MELTING RANGE – 132° to 134°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Pyrogallol Solution, Alkaline

Dissolve 0.5 g of *pyrogallol* in 2 ml of *water*; dissolve 12 g of *potassium hydroxide* in 8 ml of *water*. Mix the two solutions, immediately before use.

Quinalizarin : CI 58500; 1,2,5,8-Tetrahydroxyanthraquinone; $C_{14}H_8O_6$ = 272.21

DESCRIPTION – Reddish-brown powder.

SOLUBILITY – Insoluble in *water*; soluble in *sodium hydroxide solution*, forming a blue-violet solution; soluble in *sulphuric acid*, forming a blue solution which, on dilution, turns crimson and deposits a red precipitate.

SULPHATED ASH – Not more than 5.0 per cent, Appendix 3.2.7.

Quinhydrone : Cyclohexa-2,5-diene-1,4-dione Compound with Quinone; $C_6H_4(OH)_2.C_6H_4O_2$ = 218.21

DESCRIPTION – Dark green, lustrous crystals or crystalline powder.

SOLUBILITY – Slightly soluble in *water*; sparingly soluble in hot *water*; soluble in *alcohol*, in *solvent ether* and in *strong ammonia solution*.

MELTING RANGE – 170° to 172°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Quinidine Sulphate

Of the Indian Pharmacopoeia.

Quinine : $C_{20}H_{24}N_2O_2,3H_2O$ = 378.46

DESCRIPTION – Microcrystalline powder.

SOLUBILITY – Very slightly soluble in *water*; very soluble in *alcohol*; freely soluble in *chloroform*; sparingly soluble in *benzene*.

MELTING POINT – About 57°, Appendix 5.11.

SPECIFIC OPTICAL ROTATION – At 20°, about − 165°, determined on a 1 per cent w/v solution of the dried material in *ethyl alcohol*, Appendix 5.12.

Quinine Sulphate

Of the Indian Pharmacopoeia.

Quinoline : $\overline{CH:CH.CH:CH.C:C.CH:CH.CH:N}$ = 129.15

Contains not less than 98.5 per cent w/v of C_9H_7N.

DESCRIPTION – Clear, pale yellow liquid; odour, characteristic and unpleasant; hygroscopic.

SOLUBILITY – Soluble, with difficulty, in *water*; miscible with *alcohol*, and with *solvent ether*.

DISTILLATION RANGE – Not less than 95 per cent distils between 235° and 238°, Appendix 5.3.

REFRACTIVE INDEX – At 20°, 1.624 to 1.627, Appendix 5.14.

WT. PER ML – At 20°, 1.093 to 1.097 g, Appendix 5.19.

TARRY IMPURITIES – Dissolve 1.0 g in a mixture of 2.5 ml of *water* and 2.5 ml of *hydrochloric acid*, add 3 ml of *water* and 1 ml of *potassium chromate solution*, and heat to boiling; the solution shows no darkening or deposition of tar.

ASSAY – Weigh accurately about 0.5 g, dissolve in suitable quantity of *glacial acetic acid* by warming and cooling, if necessary, and titrate with *0.1N perchloric acid*, using *1-naphtholbenzene solution* as indicator. Each ml of *0.1N perchloric acid* is equivalent to 0.01292 g of C_9H_7N.

Quinoline Solution

Dissolve 50 ml of *quinoline* in a mixture of 60 ml of

hydrochloric acid and 300 ml of *water*; previously heated to 70°, cool and filter.

Raney Nickel Catalyst

A powdered alloy containing 40.0 to 50.0 per cent of nickel and 50.0 to 60.0 per cent aluminium.

Resorcinol : Resorcin; Benzene-1,3-diol; 1-3-dihydroxybenzene; $C_6H_4(OH)_2$ = 110.11

Contains not less than 99.0 per cent of $C_6H_6O_2$.

DESCRIPTION – Colourless or nearly colourless, needle-shaped crystals or powder; odour, slight but characteristic; taste, sweetish and then bitter. Sublimes on heating.

SOLUBILITY – Freely soluble in *water*, and in *alcohol*; soluble in *solvent ether*, and in *glycerin*.

ACIDITY – A 5 per cent w/v solution is not acid to *methyl orange solution*.

MELTING RANGE – 109° to 111°, Appendix 5.11.

CATECHOL – Dissolve 0.5 g in 10 ml of *water*, add 0.1 ml of *dilute acetic acid* and 0.5 ml of *lead acetate solution*; no turbidity is formed.

PHENOL – Warm 5 ml of a 5 per cent w/v solution in water; no odour of phenol is perceptible.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1.5 g and dissolve in *water* to make 500 ml. Transfer 25 ml of this solution to an iodine-flask, dilute with 50 ml of *water*, and add 50 ml of *0.1N bromine* and 20 ml of *dilute hydrochloric acid*. At once stopper the flask, shake and allow to stand in the dark for five minutes. Add 2 g of *potassium iodide* and 5 ml of *carbon tetrachloride* and titrate the liberated iodine with *0.1N sodium thiosulphate*, shaking thoroughly and continuing the titration until the pink colour disappears from the organic layer. Repeat the experiment with the same quantities of the same reagents in the same manner, omitting resorcinol. The difference between the titrations represents the amount of bromine required by the resorcinol. Each ml of *0.1N bromine* is equivalent to 0.001835 g of $C_6H_6O_2$.

STORAGE – Resorcinol should be kept in a well-closed container, protected from light.

Riboflavine

Of the Indian Pharmacopoeia.

Ruthenium Red : Ammoniated Ruthenium Oxychloride; $H_{42}N_{14}O_2Cl_6Ru_3$ = 786.35

DESCRIPTION – Dark brown powder.

SOLUBILITY – Completely soluble in *water*, yielding a bright crimson-red solution; soluble in *lead acetate solution*.

Ruthenium Red Solution

Dissolve 8 mg of ruthenium red in 10 ml of lead acetate solution.

Ruthenium Red Solution must be freshly prepared.

Salicylaldehyde : 2-Hydroxybenzaldehyde $HO.C_6H_4.CHO$ = 122.12

DESCRIPTION – Clear, colourless, oily liquid; odour, characteristic.

SOLUBILITY – Slightly soluble in *water*; soluble in *alcohol*

REFRACTIVE INDEX – At 20°, 1.573 to 1.574, Appendix 5.14.

WT. PER ML – At 20°, 1.164 to 1.167, Appendix 5.19.

Salicylic Acid

Of the Indian Pharmacopoeia.

Salicylic Acid Solution, Standard

A freshly prepared 0.010 per cent w/v solution of *salicylic acid* in *water*.

NOTE – Standard Salicylic Acid Solution should be freshly prepared.

Saline, Physiological

Sodium Chloride Injection of the Indian Pharmacopoeia.

Saline Solution

A 0.9 per cent w/v solution of *sodium chloride* in freshly prepared *water*, sterilised by *Heating in an Autoclave*, Appendix 4.6.

Selenium : Se = 78.96

Contains not less than 99.0 per cent of Se.

DESCRIPTION – Dark-red to greyish-black, fine powder.

MELTING RANGE – 220° to 222°, Appendix 5.11.

SOLUTION IN NITRIC ACID – 1 g dissolved in 5 ml of *nitric acid* and diluted to 50 ml with *water* yields a clear and almost colourless solution.

NON-VOLATILE MATTER – 2 g, when gently ignited, leaves not more than 2 mg of residue.

LOSS ON DRYING – When dried at 105° for one hour, loses not more than 0.1 per cent of its weight, Appendix 5.8.

ASSAY – To 0.5 g in a beaker, add 20 ml of *perchloric acid*, cover the beaker with a watch glass, and heat gently until

the metal dissolves. Cool and dilute the solution to 250 ml with *water*. To 25 ml of this solution add 100 ml of *water* and 5 g of *potassium iodide* and titrate with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.001974 g of Se.

Semicarbazide Acetate Solution

Triturate 2.5 g of *semicarbazide hydrochloride* with 3.3 g of *sodium acetate*, add 10 ml of *methyl alcohol*, mix, transfer to a flask with the aid of 20 ml of *methyl alcohol*, and allow to stand at a temperature of about 4° for thirty minutes; filter, and add sufficient *methyl alcohol* to produce 100 ml.

Semicarbazide Hydrochloride : $NH_2.CO.NH.NH_2,HCl$ = 111.53

Contains not less than 99.0 per cent of CH_5N_3O,HCl

DESCRIPTION – White to yellowish-white crystals or crystalline powder.

SOLUBILITY – Soluble in *water*; practically insoluble in *ethyl alcohol* and in *solvent ether*.

MELTING RANGE – 173° to 178°, Appendix 5.11.

HYDRAZINE – To 5 ml of a 0.2 per cent w/v solution in *water*, add 1 ml of a 2 per cent w/v solution of *dimethylaminobenzaldehyde* in *alcohol*, and 2 ml of *hydrochloric acid*; set aside for fifteen minutes. The solution shows no orange tint when compared with a solution prepared in a similar manner, omitting the semicarbazide hydrochloride.

ASSAY – Weigh accurately about 0.2 g and dissolve in 10 ml of *water*, add 30 ml of *hydrochloric acid*, and 2 ml of *chloroform*, and titrate with *0.05M potassium iodate* until the chloroform layer becomes colourless, shaking vigorously after each addition. Each ml of *0.05M potassium iodate* is equivalent to 0.005577 g of CH_5N_3O,HCl.

Silica Gel

Partially dehydrated, polymerised, colloidal silicic acid containing cobalt chloride as an indicator.

DESCRIPTION – Blue granules, becoming pink when the moisture absorption capacity is exhausted.

Silica Gel absorbs about 30 per cent of its weight of water at 20°. Its absorptive capacity may be regenerated by heating at 150° for two hours.

Silica Gel G

A fine, white, homogeneous powder of an average particle size between 10 and 40 μm containing about 13 per cent w/w of calcium sulphate hemihydrate ($CaSO_4,\frac{1}{2}H_2O$)

CALCIUM SULPHATE CONTENT – Weigh accurately about 0.25 g, and 3 ml of *2N hydrochloric acid* and 100 ml of *water* and shake vigorously for thirty minutes. Filter and wash the residue with *water*. Titrate the combined filtrate and washings with *0.05M disodium ethylenediaminetetraacetate* to within a few ml of the expected endpoint, add 4 ml of *10N sodium hydroxide* and 0.1 g of *calcon mixture* and continue the titration until the colour changes from pink to full blue. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.00726 g of $CaSO_4,\frac{1}{2}H_2O$.

ALKALINITY – pH of a suspension prepared by shaking 1 g with 10 ml of *carbon dioxide-free water* for 5 minutes, about 7, Appendix 5.10.

SEPARATING POWER – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *toluene* as the mobile phase. Apply to the plate 10 μl of a solution in *methylene chloride* containing 0.01 per cent w/v of each of *indophenol blue, sudan red G* and *dimethyl yellow* in *toluene*. Allow the mobile phase to ascend 10 cm. The chromatogram shows three clearly separated spots, the spot due to indophenol blue near the starting point, that due to dimethyl yellow in the middle of the chromatogram and that due to sudan red G between the two.

Silica Gel GF 254 : Silica Gel G/UV 254

A fine, white, homogeneous powder of an average particle size between 10 and 40 μm containing about 13 per cent w/w of calcium sulphate hemihydrate ($CaSO_4,\frac{1}{2}H_2O$) and about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at 254 nm.

It complies with the tests for **CALCIUM SULPHATE CONTENT, ALKALINITY** and **SEPARATING POWER** stated under Silica Gel G and with the following test:

FLUORESCENCE – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using a mixture of 90 volumes of *isopropyl alcohol* and 10 volumes of *anhydrous formic acid* as the mobile phase. Apply separately to the plate increasing quantities from 1 to 10 μl of a 0.1 per cent w/v solution of *benzoic acid* in the same solvent mixture. After removal of the plate dry it in a current of warm air, and examine under an ultra-violet lamp having a maximum output at about 254 nm. The benzoic acid appears as dark spots on a fluorescent background in the upper third of the chromatogram at levels of 2 μg and greater.

Silica Gel H

A fine, white, homogeneous powder of an average particle size between 10 to 40 μm.

It complies with the tests for **SEPARATING POWER** and **ALKALINITY** stated under Silica Gel G.

Silica Gel HF 254 : Silica Gel H/UV 254

A fine, white, homogeneous powder of an average

particle size between 10 and 40 µm containing about 1.5 per cent w/w of a fluorescent indicator having a maximum intensity at about 254 nm. It complies with the tests for **ALKALINITY** and **SEPARATING POWER** stated under Silica Gel G, and with the test for **FLUORESCENCE** stated under Silica Gel GF 254.

Silica Gel HF 254, Silanised

A fine, white, homogeneous powder which after shaking with water floats on the surface because of the water repellent properties. It complies with the following test :

SEPARATING POWER – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using a plate prepared in the following manner. Vigorously shake 30 g of the silica gel for two minutes with 60 ml of a mixture of 2 volumes of *water* and 1 volume of *methyl alcohol* and, using a spreading device designed for the purpose, spread a uniform layer of the suspension about 0.25 mm thick on a series of carefully cleaned glass plates measuring 20 cm × 5 cm; allow the coated plates to dry in air, and heat in an oven at 105° to 110° for thirty minutes. Use a mixture of 65 volumes of *dioxan*, 25 volumes of *water* and 10 volumes of *glacial acetic acid* as the mobile phase. Apply separately to the plate three portions, each of 10 µl, of a solution prepared in the following way: To a mixture of 0.1 g of each of *methyl laurate*, *methyl myristate*, *methyl palmitate* and *methyl stearate* add 40 ml of a decanted 3 per cent w/v solution of *potassium hydroxide* in *alcohol (90 per cent)* and heat under a reflux condenser on a water-bath for one hour. Cool, add 100 ml of *water*, acidify the mixture with *2N hydrochloric acid* and extract with three quantities, each of 10 ml, of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter and evaporate to dryness. Dissolve the residue in 50 ml of *chloroform*. After removal of the plate dry it by heating in an oven at 120° for thirty minutes. Allow to cool, and spray with a 3.5 per cent w/v solution of *phosphomolybdic acid* in *isopropyl alcohol* and heat at 150° until the spots become visible. Expose the plate to ammonia vapour until the background turns white; each chromatogram shows four clearly separated spots.

Silicotungstic Acid : 12-Tungstosilicic Acid
$SiO_2,12WO_3,xH_2O$

Contains not less than 85.0 per cent of WO_3 and not less than 1.85 per cent of SiO_2.

DESCRIPTION – Colourless crystals or a crystalline powder.

SOLUBILITY – Freely soluble in *water*, and in *alcohol*.

CHLORIDE – 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with *limit test for sulphates*, Appendix 3.2.8.

TUNGSTATE – Dissolve 0.5 g in 5 ml of *water*, filter and add 5 drops of *dilute hydrochloric acid*; no turbidity is produced.

ASSAY – *For* WO_3 – Dry a small quantity, accurately weighed, by gentle ignition, and weigh; transfer about 1 g of the dried material, accurately weighed, to a silica boat and place it in a short silica tube; ignite at red heat in a stream of *gaseous dry hydrochloric acid* and air until the residue is white. The loss in weight represents the amount WO_3 in the dried material; calculate the amount of WO_3 in the original material.

For SiO_2 – Transfer the residue from the **ASSAY** for WO_3 to a platinum dish, and weigh; evaporate with the addition of *hydrofluoric acid*; cool, and reweigh. The loss in weight represents the amount of SiO_2 in the quantity of dried material taken in the **ASSAY** for WO_3; calculate the amount of SiO_2 in the original material.

Silicotungstic Acid Solution

A 10.0 per cent w/v solution of *silicotungstic acid* in *water*.

Silver Nitrate

Of the Indian Pharmacopoeia.

Silver Nitrate-Pyridine Reagent : Silver Nitrate Solution in Pyridine

An 8.5 per cent w/v solution of *silver nitrate* in *pyridine*.

Silver Nitrate Solution

A 5.0 per cent w/v solution of *silver nitrate* in *water*.

Silver Nitrate Solution, Alcoholic

Dissolve 4 g of *silver nitrate* in 10 ml of *water*, and add sufficient quantity of *alcohol* to produce 100 ml.

Silver Nitrate Solution, Ammoniacal : Silver Ammonio-nitrate Solution.

Dissolve 2.5 g of *silver nitrate* in 80 ml of *water* and cautiously add *dilute ammonia solution* until the precipitate first formed is nearly dissolved; set aside, decant and add sufficient *water* to produce 100 ml.

CAUTION – Dry ammoniacal silver nitrate is very explosive.

Silver Nitrate Solution, Methanolic

Boil, until dissolved, 5 g of *silver nitrate* with 60 ml of *methyl alcohol* under reflux on a water-bath. Use the solution while still hot.

Silver Oxide : Ag_2O = 231.74

DESCRIPTION – Brownish-black, heavy, odourless powder.

SOLUBILITY – Practically insoluble in *water*; freely soluble in *dilute nitric acid*, and in *strong ammonia*; insoluble in *alcohol*.

LOSS ON DRYING – Loses not more than 0.25 per cent of its weight, when dried at 120° for three hours, Appendix 5.8.

STORAGE – Store in well-closed containers; do not expose to ammonia fumes or easily oxidizable substances.

Soda Lime

Soda Lime is a mixture of sodium hydroxide, or sodium hydroxide and potassium hydroxide, with calcium hydroxide. It absorbs not less than 20.0 per cent of its weight of carbon dioxide. It may contain an indicator which is inert with respect to its reactivity with solvent ether, ethylene, cyclopropane, and nitrous oxide, and which changes colour when the absorption capacity of the soda lime for carbon dioxide is exhausted.

DESCRIPTION – White or greyish-white granules, or it may be coloured if any indicator is added.

SOLUBILITY – Partially soluble in *water*; almost completely soluble in *dilute acetic acid*.

SIZE AND HARDNESS OF GRANULES – Shake 500 g on a 8 mm perforated plate test sieve; the whole passes through. Then shake on a *No. 6 sieve*; not more than 25 g passes through. Place the screened material in a round tin, about 12.5 cm high and 10 cm in diameter; secure the lid and allow to roll slowly down an inclined plane, 274.5 cm long, with the lower end raised 30.5 cm above a wooden base so that at the end of its roll the tin drops through 30.5 cm on to the wood. Repeat this operation 20 times and then shake the contents of the tin on a *No.8 sieve*, not more than 25 g passes through sieve.

LOSS ON DRYING – Loses not less than 15 per cent and not more than 19 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

MOISTURE ABSORPTION – Weigh accurately about 10 g and place in an open glass dish, about 50 mm in diameter and 30 mm high, in a desiccator over *sulphuric acid (14 per cent v/v)* and allow to remain for twenty-four hours; the increase in weight is not more than 7.5 per cent.

CARBON DIOXIDE ABSORPTION – Plug with cotton wool one end of a straight glass tube about 12 cm long and 1 cm internal diameter. Introduce into it about 2 g of the sample, accurately weighed, and close the free end with a plug of cotton wool. Connect by means of a short wide rubber tube to a similar tube packed with *anhydrous calcium chloride* and close both ends of the apparatus wit rubber caps and weigh the complete apparatus. Pass a slow stream, about 3 bubbles per second, of *carbon dioxide* mixed with an approximately equal volume of air for about 90 minutes, first through a wash bottle containing *sulphuric acid*, then through the soda lime and *calcium chloride* in the prepared tube in order, and finally through another wash bottle containing *sulphuric acid*. Disconnect the supply of carbon dioxide, aspirate a little air through the apparatus, allow to attain laboratory temperature, disconnect from the wash bottles, fit on the rubber caps, and weigh. The increase in weight is not less than 20.0 per cent of the amount taken for test.

STORAGE – Soda Lime should be kept in well-closed containers.

Sodium : Na = 22.99

DESCRIPTION – A soft metal, the freshly cut surface of which is bright silver-grey. It rapidly tarnishes in contact with the atmosphere and is eventually oxidised completely to sodium hydroxide and changed to sodium carbonate.

SOLUBILITY – Reacts violently with *water*, taking fire, yielding hydrogen and a solution of sodium hydroxide; soluble in *alcohol*, yielding hydrogen and a solution of sodium ethoxide; insoluble in *solvent ether*, and in *light petroleum (boiling range, 40° to 60°)*.

CHLORIDE – Dissolve 0.5 g in 25 ml of *alcohol* and add 25 ml of *water* and 3 ml of *nitric acid*; the solution complies with the *limit test for chlorides*, Appendix 3.2.2.

Sodium Acetate

Of the Indian Pharmacopoeia.

Sodium Acetate, 0.02 M

Dissolve 2.72 g of *sodium acetate* in sufficient *water* to produce 1000 ml.

Sodium Acetate, 0.1 M

Dissolve 13.61 g of *sodium acetate* in sufficient *water* to produce 1000 ml.

Sodium Acetate, M

Dissolve 136.1 g of *sodium acetate* in sufficient *water* to produce 1000 ml.

Sodium Acetate, Anhydrous : $CH_3.CO_2Na = 82.03$

Contains not less than 98.0 per cent of $C_2H_3NaO_2$.

DESCRIPTION – White, pale-grey, or very pale-brown, crystalline masses; hygroscopic.

Sodium Acid Citrate : $C_6H_6Na_2O_7,1\frac{1}{2}H_2O = 263.1$

Contains not less than 98.0 per cent and not more than the equivalent of 104.0 per cent of $C_6H_6Na_2O_7,1\frac{1}{2}H_2O$.

DESCRIPTION – White powder; odourless; taste, saline.

SOLUBILITY – Freely soluble in *water*; insoluble in *alcohol*.

ACIDITY – pH of a 3.0 per cent w/v solution, 4.9 to 5.2, Appendix 5.10.

CHLORIDE – 1 g complies with *limit test for chlorides*, Appendix 3.2.2.

OXALATE – Complies with the test described under Potassium Citrate.

ASSAY – Heat 2 g until carbonised, cool, and boil the residue with 50 ml of *water* and 50 ml of *0.5N hydrochloric acid*. Filter, wash the filter with *water*; and titrate the excess of acid in the filtrate and washings with *0.5N sodium hydroxide* using *methyl orange solution* as indicator. Each ml of a *0.5N hydrochloric acid* is equivalent to 0.06578 g of $C_6H_6Na_2O_7,1\frac{1}{2}H_2O$.

Sodium Aminosalicylate

Of the Indian Pharmacopoeia.

Sodium Bicarbonate

Of the Indian Pharmacopoeia.

Sodium Bicarbonate Solution

A 5 per cent w/v solution of *sodium bicarbonate* in *water*.

Sodium Bisulphite

Consists of sodium bisulphite ($NaHSO_3$) and sodium metabisulphite ($Na_2S_2O_5$) in varying proportions. It yields not less than 58.5 per cent and not more than 67.4 per cent of SO_2.

DESCRIPTION – White or yellowish-white crystals or granular powder; odour, of sulphur dioxide. It is unstable in air.

SOLUBILITY – Freely soluble in *water*; slightly soluble in *alcohol*.

ASSAY – Weigh accurately about 0.2 g and transfer to a glass-stoppered flask, add 50 ml of *0.1N iodine* and insert the stopper of the flask. Allow to stand for five minutes, add 1 ml of *hydrochloric acid*, and titrate the excess of iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator added towards the end of the titration. Each ml of *0.1N iodine* is equivalent to 0.003203 g of SO_2.

STORAGE – Preserve Sodium Bisulphite in tightly-closed containers in a cool place.

Sodium Bisulphite Solution

Dissolve 10 g of *sodium bisulphite* in sufficient *water* to make 30 ml.

Sodium Bisulphite Solution must be freshly prepared.

Sodium Carbonate : $Na_2CO_3,10H_2O$ = 286.15

Contains not less than 99.0 per cent and not more than the equivalent of 105.0 per cent of $Na_2CO_3,10H_2O$.

DESCRIPTION – Transparent, colourless, rhombic crystals, odourless; taste strongly alkaline; efflorescent.

SOLUBILITY – Freely soluble in *water*; practically insoluble in *alcohol*.

IRON – 2 g dissolved in 20 ml of water and 2 ml of *iron-free hydrochloric acid* and diluted to 40 ml with *water*, complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – 0.5 g, dissolved in *water* with the addition of 2 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g, dissolved in *water* with the addition of 2 ml of *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 2 g, dissolve in 20 ml of *water* and titrate with *0.5N sulphuric acid*, using *bromophenol blue solution* as indicator. Each ml of *0.5N sulphuric acid* is equivalent to 0.07154 g of $Na_2CO_3,10H_2O$.

Sodium Carbonate, Anhydrous : Na_2CO_3 = 105.99

Contains not less than 98.0 per cent of Na_2CO_3.

DESCRIPTION – White powder.

SOLUBILITY – Slowly soluble in *water*.

IRON – 4 g, dissolved in 25 ml of *water* and 8 ml of *iron-free hydrochloric acid*, complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – 5 g, with an additional 6 ml of *nitric acid*, complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – Dissolve 2 g in 3 ml of *hydrochloric acid* and 50 ml of *water*, add 1 ml of *barium chloride solution* and allow to stand for one hour; no turbidity is produced.

ASSAY – Weigh accurately about 2 g, dissolve in 50 ml of *water* and titrate with *N hydrochloric acid*, using *bromophenol blue solution* as indicator. At the first colour change, boil the solution, cool and complete the titration. Each ml of *N hydrochloric acid* is equivalent to 0.05299 g of Na_2CO_3.

Sodium Carbonate Monohydrate : Na_2CO_3, H_2O = 124.00

Contains not less than 99.5 per cent of Na_2CO_3, when dried at 105° for four hours.

DESCRIPTION – Colourless Crystals or a white crystalline powder.

SOLUBILITY – Freely soluble in *water*.

LOSS ON DRYING – Loses not less than 12 per cent and not more than 15 per cent of its weight when dried at 105° for four hours, Appendix 5.8.

ASSAY – Carry out the **ASSAY** described under anhydrous sodium carbonate.

Each ml of a *N hydrochloric acid* is equivalent to 0.05299 g of Na_2CO_3.

Sodium Carbonate Solution

A 10.0 per cent w/v solution of *sodium carbonate* in *water*.

Sodium Chloride

Of the Indian Pharmacopoeia.

Sodium Chloride Solution, Isotonic

A 0.9 per cent w/v solution of *sodium chloride* in *water*.

Sodium Chloride Injection

Of the Indian Pharmacopoeia.

Sodium Chloride Solution : Brine

A saturated solution of *sodium chloride* in *water*.

Sodium Citrate

Of the Indian Pharmacopoeia.

Sodium Cobaltinitrite : $Na_3Co(NO_2)_6$ = 403.94

DESCRIPTION – An orange-yellow powder.

SOLUBILITY – Readily soluble in *water*, forming a clear orange-red solution.

POTASSIUM – Dissolve 3 g in 10 ml of *water*, add the solution to a mixture of 5 ml of *water* and 2 ml of *dilute acetic acid*, and allow to stand for one hour; no precipitate is produced.

SENSITIVITY – To the solution obtained in the test for **POTASSIUM**, add 0.1 ml of a 1.0 per cent w/v solution of *potassium chloride* and allow to stand; a distinct precipitate is produced.

Sodium Cobaltinitrite Solution

A 30 per cent w/v solution of *sodium cobaltinitrite* in *water*.

Sodium Diethyldithiocarbamate
$(C_2H_5)_2N{\cdot}CS{\cdot}SNa,3H_2O$ = 225.30

DESCRIPTION – White or colourless crystals.

SOLUBILITY – Readily soluble in *water*, yielding a colourless solution.

SENSITIVITY – Add 10 ml of a 0.1 per cent w/v solution to 50 ml of *water* containing 0.002 mg of copper previously made alkaline with *dilute ammonia solution*. A yellowish-brown colour should be apparent in the solution when compared with a blank test containing no copper.

Sodium Diethyldithiocarbamate Solution

A 0.1 per cent w/v solution of *sodium diethyldithiocarbamate* in *water*.

Sodium Dihydrogen Phosphate

Sodium Acid Phosphate of the Indian Pharmacopoeia.

Sodium Fluoride : NaF = 41.99

Contains not less than 98.0 per cent of NaF, calculated with reference to the dried substance.

DESCRIPTION – White powder, odourless.

SOLUBILITY – Soluble in *water*; practically insoluble in *alcohol*.

ACIDITY OR ALKALINITY – Dissolve 1.0 g in 20 ml of *water* in a platinum dish, dissolve 3 g of *potassium nitrate* in the solution, and cool to 0° The solution requires not more than 2.0 ml of *0.05N sodium hydroxide* or not more than 0.5 ml of *0.05N sulphuric acid* for neutralisation to *phenolphthalein solution*.

FLUOROSILICATE – Heat to boiling the solution obtained in the test for **ACIDITY OR ALKALINITY** and titrate while hot with *0.05N sodium hydroxide* until a permanent pink colour is produced; not more than 1.5 ml of *0.05N sodium hydroxide* is required.

LEAD – Not more than 20 parts per million, Appendix 3.2.6.

LOSS ON DRYING – When dried to constant weight at 130°, loses not more than 0.5 per cent of its weight, Appendix 5.8.

ASSAY – Weigh accurately about 80 mg and dissolve in 45 ml of *water*, add 0.2 g of *sodium chloride* and 20 ml of *alcohol*, heat to boiling, and add drop-wise at first and then more rapidly, with constant stirring, 50 ml of *0.05M lead nitrate*. Continue the heating to coagulate the precipitate, allow to cool to about 20°, filter and wash the residue three times with small volumes of *alcohol (20 per cent)*. To the combined filtrate and washings, add 1 g of *hexamine* and titrate the excess of lead nitrate with *0.05M disodium ethylenediaminetetraacetate*, using *xylenol orange solution* as indicator and continuing the titration until the solution becomes yellow. Each ml of *0.05M lead nitrate* is equivalent to 0.002099 g of NaF.

STORAGE – Sodium Fluoride should be kept in well-closed containers.

Sodium Hexametaphosphate : Chiefly $(NaPO_3)_6$

DESCRIPTION – Colourless, transparent flakes or platelets.

SOLUBILITY – Slowly soluble in *water*.

ACIDITY OR ALKALINITY – A 2.0 per cent w/v solution in *carbon dioxide-free water* gives with *bromothymol blue solution* a yellow or green colour.

Sodium Hexametaphosphate Solution

Dissolve 9 g of *sodium hexametaphosphate* and 2 g of *sodium carbonate* in sufficient *water* to produce 100 ml.

Sodium Hyaluronate

See **ASSAY** of hyaluronidase, Appendix 2.6.

Sodium Hydroxide

Of the Indian Pharmacopoeia.

Sodium Hydroxide, *x* N

Solutions of any normality, *x*N may be prepared by dissolving 40*x* g of *sodium hydroxide* in water and diluting to 1000 ml.

Sodium Hydroxide, Methanolic, 0.1 N

Dissolve 4.0 g of *sodium hydroxide* in sufficient *methyl alcohol* to produce 1000 ml.

Sodium Hydroxide Solution

A 20.0 per cent w/v solution of *sodium hydroxide* in *water*.

Sodium Hydroxide Solution, Dilute

A 5.0 per cent w/v solution of *sodium hydroxide* in *water*.

Sodium Hydrosulphite : Sodium Dithionite; $Na_2S_2O_4$ = 174.10

Contains not less than 80.0 per cent of $Na_2S_2O_4$.

DESCRIPTION – A white or slightly grey, crystalline powder.

SOLUBILITY – Soluble in *water*, yielding a solution which rapidly absorbs oxygen and becomes turbid.

SUITABILITY FOR RIBOFLAVINE ASSAY – To each of two or more tubes, add 10 ml of *water* and 1.0 ml of a standard riboflavine solution containing 20 µg of riboflavine per ml, and mix. To each tube add 1.0 ml of *glacial acetic acid*, mix and, with mixing, 0.5 ml of a 4.0 per cent w/v solution of *potassium permanganate*, and allow to stand for two minutes. Then to each tube add, with mixing, 0.5 ml of *hydrogen peroxide solution*; the permanganate colour is destroyed within ten seconds. Shake the tubes vigorously until excess oxygen is expelled. Measure the fluorescence of the solution. Then add, with mixing, 8.0 mg of the sodium hydrosulphite; the riboflavine is completely reduced in not more than five seconds.

ASSAY – Dissolve 1 g in 10 ml of *formaldehyde solution*, previously neutralised to *phenolphthalein solution*, and contained in a 25-ml glass-stoppered vessel, nearly fill the vessel with previously boiled and cooled *water*, insert the stopper, mix and allow to stand for twenty minutes. Transfer to a 250-ml flask, add 100 ml of previously boiled and cooled *water*, neutralise by the addition of *N sulphuric acid*, using a few drops of *methyl orange solution* as indicator, add sufficient previously boiled and cooled *water* to produce 250 ml, and mix. Neutralise 25 ml of this solution with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator, and titrate with *0.1N iodine*, using *starch solution* as indicator; destroy the blue colour by the addition of 1 drop of *0.1N sodium thiosulphate* and titrate with *0.1N sodium hydroxide* to a pink colour. Each ml of *0.1N sodium hydroxide* is equivalent to 0.003482 g of $Na_2S_2O_4$.

STORAGE – Sodium Hydrosulphite must be protected from atmospheric oxidation.

Sodium Hypobromite Solution

In a bath of iced *water* mix 20 ml of *10N sodium hydroxide* and 500 ml of *water*, add 5 ml of *bromine solution* and stir gently until solution is complete.

Prepare Sodium Hypobromite Solution immediately before use.

Sodium Hypobromite Solution, Alkaline

Dissolve 10 g of *sodium hydroxide* in 400 ml of *water*, add 5.5 ml of *bromine*, stir to dissolve, and add sufficient *water* to produce 500 ml. Ascertain the strength of the solution by adding to 10 ml, 25 ml of *water*, 2 g of *potassium iodide*, and 10 ml of *glacial acetic acid*, and titrating the liberated iodine with *0.1N sodium thiosulphate*; each ml of *0.1N sodium thiosulphate* is equivalent to 0.008 g of available bromine. Dilute the remainder of the solution to contain 1.5 per cent w/v of available bromine; to 66 ml of the resulting solution add 20 ml of *sodium hydroxide solution* and sufficient *water* to produce 100 ml.

Sodium Hypochlorite Solution

Contains not less than 10.0 per cent w/v and not more than 16.0 per cent w/v of available chlorine.

DESCRIPTION – Clear, colourless to pale-yellow liquid having an odour of chlorine. It is affected by light and gradually deteriorates.

ASSAY – Transfer 1 ml in a glass-stoppered flask containing a solution of 3 g of *potassium iodide* in 100 ml of *water*, add 20 ml of *dilute acetic acid* and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator added towards the end of the titration. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003546 g of available chlorine.

STORAGE – Store Sodium Hypochlorite Solution in light-resistant containers, preferably below 25°.

Sodium Hypochlorite Solution, Dilute

Dilute 35 ml of *sodium hypochlorite solution* to 100 ml with *water* immediately before use.

Dilute Sodium Hypochlorite Solution contains approximately 3.5 per cent w/v of available chlorine.

Sodium Hypophosphite : NaH_2PO_2,H_2O = 106.00

DESCRIPTION – Crystalline powder or colourless crystals; hygroscopic.

SOLUBILITY – Freely soluble in *water*; soluble in *alcohol*.

Sodium Lauryl Sulphate

Of the Indian Pharmacopoeia.

Sodium Lauryl Sulphate, Purified

Contains not less than 99.0 per cent of sodium alkyl sulphates, calculated as $C_{12}H_{25}OSO_3Na$ and determined on the dried substance.

DESCRIPTION – White, crystalline flakes.

SOLUBILITY – Soluble in *water*.

COLOUR AND CLARITY OF SOLUTION – A 10 per cent w/v solution remains clear and colourless when allowed to stand for seven days.

LOSS ON DRYING – When dried at 105° for two hours, loses not more than 2.0 per cent of its weight, Appendix 5.8.

ASSAY – To 5.0 g, previously dried at 105° to constant weight, add 25.0 ml of *N sulphuric acid* and heat under a reflux condenser for two hours. Cool, rinse the condenser with 30 ml of *ethyl alcohol* and then with *water*, and titrate the excess of *sulphuric acid* with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.2884 g of sodium alkyl sulphates, calculated as $C_{12}H_{25}OSO_3Na$.

Sodium Metabisulphite : Sodium Pyrosulphite

Of the Indian Pharmacopoeia.

Sodium Molybdate : $Na_2MoO_4,2H_2O$ = 241.95

Contains not less than 58.0 per cent of MoO_3.

DESCRIPTION – Colourless crystals or a white, crystalline powder.

SOLUBILITY – Soluble in *water*.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

PHOSPHATE – Dissolve 1 g in 15 ml of *water* and 5 ml of *dilute ammonia solution*. Pour into 25 ml of *dilute nitric acid*, and allow to stand at 40° for one hour; no yellow precipitate is produced.

SULPHATE – 1 g, with the addition of 1.5 ml of *hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

AMMONIA – Add 1 g to 5 ml of *sodium hydroxide solution* and boil; no ammonia is evolved.

ASSAY – Carry out the **ASSAY** described under Molybdic Acid.

Sodium Molybdophosphotungstate Solution

Boil for two hours in a flask fitted with a reflux condenser, 350 ml of *water*, 50 g of *sodium tungstate*, 12 g of *phosphomolybdic acid* and 25 ml of *phosphoric acid*; cool and add sufficient *water* to produce 500 ml.

Sodium 1,2-Naphthaquinone-4-Sulphonate

$C_{10}H_5NaO_5S$ = 260.20

DESCRIPTION – Yellow or orange, crystalline powder.

SOLUBILITY – Soluble in *water*; insoluble in *alcohol*.

Sodium Nitrate : $NaNO_3$ = 84.99

Contains not less than 98.0 per cent of $NaNO_3$.

DESCRIPTION – Colourless crystals or a white, granular powder.

SOLUBILITY – Soluble in *water*; slightly soluble in *glycerin*.

CHLORIDE – 3 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 2 g complies with the *limit test for sulphates* Appendix 3.2.8.

IRON – 2 g complies with the *limit test for iron*, Appendix 3.2.5.

HEAVY METALS – Dissolve 1 g in 15 ml of *water*, add 2 ml of *dilute acetic acid* and *water* to produce 25 ml; the limit of heavy metals is 20 parts per million, Appendix 3.2.4.

ASSAY – Carry out the **ASSAY** described under Potassium Nitrate. Each ml of *0.1N hydrochloric acid* is equivalent to 0.008499 g of $NaNO_3$.

Sodium Nitrite : $NaNO_2$ = 68.99

Contains not less than 97.0 per cent and not more than 101.0 per cent of $NaNO_2$, calculated with reference to the substance dried over *silica gel* for four hours.

DESCRIPTION – Colourless to slightly yellow crystals or a white or slightly yellow, granular powder; odourless; taste, saline; deliquescent.

SOLUBILITY – Freely soluble in *water*; sparingly soluble in *alcohol*.

CHLORIDE – 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

LOSS ON DRYING – Loses not more than 0.25 per cent of its weight, when dried over *silica gel* for four hours, Appendix 5.8.

ASSAY – Weigh accurately about 1 g and dissolve in water to make 100 ml. Add by a pipette 10 ml of this solution to a mixture of 50 ml of *0.1N potassium permanganate*, 100 ml of *water* and 5 ml of *sulphuric acid* immersing the tip of the pipette beneath the surface of the mixture during the addition. Warm the liquid to 40°, allow it to stand for five minutes, and add 25 ml of *0.1N oxalic acid*, heat the mixture to about 80° and titrate with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.00345 g of $NaNO_2$.

Sodium Nitrite Solution

A 10 per cent w/v solution of *sodium nitrite* in *water*.

Sodium Nitroprusside : $Na_2[Fe(CN)_5(NO)],2H_2O$ = 297.95

DESCRIPTION – Ruby-red crystals.

SOLUBILITY – Readily soluble in *water*.

FERRICYANIDE – Dissolve 1 g in 10 ml of *water*, and add 1 ml of a 10 per cent w/v solution of *ferrous sulphate*; no blue colour is produced.

FERROCYANIDE – Dissolve 1 g in 10 ml of *water* and add 0.2 ml of *ferric chloride solution*; no blue colour is produced.

Sodium Nitroprusside Solution

A 1.0 per cent w/v solution of *sodium nitroprusside* in *water*.

Sodium Nitroprusside Solution should be recently prepared.

Sodium Nitroprusside Solution, Alkaline

Dissolve 1 g of *sodium nitroprusside* and 1 g of *sodium carbonate* in sufficient *water* to produce 100 ml.

Sodium Oxalate : $C_2O_4Na_2$ = 134.00

Contains not less than 99.5 per cent of $C_2O_4Na_2$.

DESCRIPTION – White, crystalline powder.

SOLUBILITY – Slightly soluble in *water* yielding a clear colourless solution.

ACIDITY OR ALKALINITY – 2 g, dissolved in 100 ml of *water*, requires for neutralisation not more than 0.1 ml of *0.1N sodium hydroxide* or 0.1 ml of *0.1N hydrochloric acid*, using *phenolphthalein solution* as indicator.

ASSAY – Weigh accurately about 0.3 g, dissolve in 25 ml of *water*, and add 6 ml of *sulphuric acid*, previously diluted with a little *water*. Titrate the solution at a temperature of about 70° with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.00670 g of $C_2O_4Na_2$.

Sodium Periodate : Sodium Metaperiodate; $NaIO_4$ = 213.89

Contains not less than 98.0 per cent of $NaIO_4$.

DESCRIPTION – White crystals or a white crystalline powder.

SOLUBILITY – Soluble in *water*.

MANGANESE – Boil 1 g with 40 ml of *water* and 10 ml of *sulphuric acid* for one minute, and allow to stand for five minutes; no pink colour is produced.

BROMIDE, CHLORATE AND CHLORIDE – Dissolve 1 g in a mixture of 50 ml of *water* and 2 ml of *nitric acid*, cool and add 0.1 g of *sodium nitrite* and 1 ml of *silver nitrate solution*. Any opalescence produced is not greater than the standard opalescence used in the *limit test for chlorides*, Appendix 3.2.2.

IODIDE – Dissolve 1 g in 20 ml of *water* and 10 ml of *dilute sulphuric acid*, add 0.5 ml of *chloroform*, shake thoroughly, and allow to separate; the chloroform layer does not become pink or violet.

ASSAY – Dissolve about 0.5 g, accurately weighed, in 100 ml of *water*, add 3 g of *sodium bicarbonate* and 3 g of *potassium iodide*, and titrate the liberated iodine with *0.1N sodium arsenite*. Each ml of *0.1N sodium arsenite* is equivalent to 0.01069 g of $NaIO_4$.

Sodium Peroxide : Na_2O_2 = 77.98

Contains not less than 85.0 per cent of Na_2O_2.

DESCRIPTION – Yellowish powder or granules; hygroscopic.

SOLUBILITY – Readily soluble in *water*, with evolution of oxygen.

ASSAY – Weigh accurately about 0.2 g into a dry flask, add a mixture of 10 ml of *potassium iodide solution*, 10 ml of *dilute hydrochloric acid* and 10 ml of *water*, and titrate the liberated iodine with *0.1N sodium thiosulphate*. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003899 g of Na_2O_2.

Sodium Phosphate

See *disodium hydrogen phosphate*.

Sodium Picrate-Picric Acid Solution

Add 0.5 ml of *sodium hydroxide solution* to 100 ml of a saturated solution of *picric acid* in *water*.

Sodium Potassium Tartrate : Rochelle Salt
$COONa{\cdot}CH(OH){\cdot}CH(OH){\cdot}COOK,4H_2O = 282.17$

Contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of $C_4H_4O_6KNa,4H_2O$.

DESCRIPTION – Colourless crystals or a white, crystalline powder; odourless; taste saline and cooling. As it effloresces slightly in warm, dry air, the crystals are often coated with a white powder.

SOLUBILITY – Soluble in *water;* practically insoluble in *alcohol*.

ACIDITY OR ALKALINITY – Dissolve 1 g in 10 ml of recently boiled and cooled *water*; the solution requires for neutralisation not more than 0.1 ml of *0.1N sodium hydroxide* or of *0.1N hydrochloric acid*, using *phenolphthalein solution* as indicator.

IRON – 0.5 g complies with the *limit test for iron*, Appendix 3.2.5.

CHLORIDE – 0.5 g complies with the *limit test for chlorides*, Appendix 3.2.2.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 2 g and heat until carbonised, cool, and boil the residue with 50 ml of *water* and 50 ml of *0.5N sulphuric acid*; filter, and wash the filter with *water*; titrate the excess of acid in the filtrate and washings with *0.5N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *0.5N sulphuric acid* is equivalent to 0.07056 g of $C_4H_4O_6KNa,4H_2O$.

Sodium Pyrophosphate : $Na_4P_2O_7,10H_2O = 446.06$

Contains not less than 99.0 per cent of $Na_4P_2O_7,10H_2O$.

DESCRIPTION – Colourless crystals.

SOLUBILITY – Readily soluble in *water*.

ALKALINITY – pH of a 5 per cent w/v solution, about 10.7, Appendix 5.10.

ASSAY – Weigh accurately about 3 g, dissolve in 50 ml of *water* and titrate with *N hydrochloric acid*, using *bromocresol green solution* as indicator, titrating to the green colour indicative of pH 4.5. Each ml of *N hydrochloric acid* is equivalent to 0.2230 g of $Na_4P_2O_7,10H_2O$.

Sodium Salicylate

Of the Indian Pharmacopoeia.

Sodium Salicylate Solution

A 10.0 per cent w/v solution of *sodium salicylate* in *water*.

Sodium Silicate : Na_2SiO_3,xH_2O

DESCRIPTION – White powder.

SOLUBILITY – Completely soluble in cold *water*.

LOSS ON IGNITION – When gently ignited to constant weight, loses not less than 35.0 per cent and not more than 50.0 per cent of its weight.

Sodium Silicotungstate Solution

Boil gently 0.60*b* g of *silicotungstic acid* and 10 g of *sodium chloride* with 0.24*ab* ml of *0.1N sodium hydroxide* for ten minutes. Cool and dilute the solution to 100 ml with *water*. The values for *a* and *b* are determined in the following manner:

Dissolve 3 g of *silicotungstic acid* in 30 ml of *water* and titrate with *0.1N sodium hydroxide* to a permanent yellow colour, using *thymol blue solution* as indicator. Record the burette reading (*a* ml).

Dissolve 0.463 g of *procaine hydrochloride* and 0.606 g of *benzylpenicillin sodium* in sufficient *water* to produce 500 ml. Prepare a solution of sodium silicotungstate by dissolving 2.5 g *silicotungstic acid* in 1.15 ml of *0.1N sodium hydroxide* and diluting to 50 ml with *water*. To 50 ml of the procaine penicillin solution add 1 g of *sodium chloride* and titrate with the sodium silicotungstate solution, with constant stirring, until the precipitate coagulates. Then continue the titration, adding 0.1 ml of the titrant at a time. After each addition, stir well, allow the precipitate to settle, transfer 0.5 ml of the clear supernatant liquid to a test tube and add 0.5 ml of *0.1N iodine*. If a turbidity is produced continue the titration until the supernatant liquid gives no turbidity with *0.1N iodine*. Repeat the titration using a further 50 ml of the procaine penicillin solution containing 1 g of *sodium chloride* and adding, before testing with iodine, the volume of the sodium silicotungstate solution required in the first titration. Record the burette reading of the second titration (*b* ml).

Sodium Solution FP

Dissolve 0.5084 g of *sodium chloride*, previously dried to constant weight at 130°, in sufficient freshly distilled *water* to produce 1000.0 ml.

Sodium Solution FP contains 200 µg of Na per ml.

Sodium Sulphate, Anhydrous : $Na_2SO_4 = 142.04$

DESCRIPTION – White, crystalline powder or granules; hygroscopic.

SOLUBILITY – Freely soluble in *water*; practically insoluble in *alcohol*.

ACIDITY OR ALKALINITY – 4 g, dissolved in 100 ml of *carbon dioxide-free water*, requires for neutralisation to the green colour of *bromothymol blue solution* indicative of pH 7; not more than 0.5 ml of either *0.1N sodium hydroxide* or *0.1N hydrochloric acid*.

Sodium Sulphide : $Na_2S,9H_2O$ = 240.18

Contains not less than 95.0 per cent of $Na_2S,9H_2O$.

DESCRIPTION – Moist, colourless crystals.

SOLUBILITY – Readily soluble in *water* forming a clear colourless solution.

ALKALINITY – A solution in *water* is strongly alkaline.

ASSAY – Weigh accurately about 0.5 g, dissolve in 30 ml of *carbon dioxide-free water* in a glass-stoppered flask, add with constant shaking, 50 ml of *0.1N iodine*, followed by 2 ml of *hydrochloric acid* and titrate the excess of iodine with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N iodine* is equivalent to 0.01201 g of $Na_2S,9H_2O$.

Sodium Sulphide Solution

A 10.0 per cent w/v solution of *sodium sulphide* in *water*.

Sodium Sulphite : $Na_2SO_3,7H_2O$ = 252.15

Contains not less than 96.0 per cent of $Na_2SO_3,7H_2O$.

DESCRIPTION – White or colourless crystals.

SOLUBILITY – Freely soluble in *water*; slightly soluble in *alcohol*.

FREE ACID – Dissolve 1 g in 10 ml of *water* and add 2 drops of *phenolphthalein solution*; a pink colour is produced.

ASSAY – To about 0.5 g, accurately weighed, add 50 ml of *0.1N iodine* and allow to stand for five minutes. Add 1 ml of *hydrochloric acid*, and titrate with *0.1N sodium thiosulphate*, using *starch solution* as indicator. Each ml of *0.1N iodine* is equivalent to 0.01261 g of $Na_2SO_3,7H_2O$.

Sodium Sulphite, Anhydrous : Na_2SO_3 = 126.06

DESCRIPTION – Small crystals or powder.

SOLUBILITY – Freely soluble in *water*; soluble in *glycerin*; almost insoluble in *alcohol*.

Sodium Tartrate : $C_4H_4O_6Na_2,2H_2O$ = 230.08

DESCRIPTION – Transparent crystals.

SOLUBILITY – Very soluble in *water*, practically insoluble in *alcohol*.

LOSS ON DRYING – Not less than 15.6 per cent and not more than 15.7 per cent, determined by heating in an oven at 150°, Appendix 5.8.

Sodium Thiosulphate

Of the Indian Pharmacopoeia.

Sodium Tungstate : $Na_2WO_4,2H_2O$ = 329.86

DESCRIPTION – Colourless crystals or a white crystalline powder.

SOLUBILITY – Readily soluble in *water* forming a clear solution.

ALKALINITY – pH of a 2 per cent w/v solution in *carbon dioxide-free water*, 8.0 to 9.0, Appendix 5.10.

NITRATE – Dissolve 1 g in 10 ml of *water*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the blue colour is not entirely discharged.

LOSS ON IGNITION – When ignited to constant weight, loses not less than 10.5 per cent and not more than 11.5 per cent of its weight.

Stannous Chloride : $SnCl_2,2H_2O$ = 225.63

Contains not less than 97.0 per cent of $SnCl_2,2H_2O$.

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *dilute hydrochloric acid*.

ARSENIC – Dissolve 5.0 g in 10 ml of *hydrochloric acid*, heat to boiling and allow to stand for one hour; the solution shows no darkening when compared with a freshly prepared solution of 5.0 g in 10 ml of *hydrochloric acid*.

SULPHATE – 5.0 g, with the addition of 2 ml of *dilute hydrochloric acid*, complies with the *limit test for sulphates*, Appendix 3.2.8.

ASSAY – Weigh accurately about 1.0 g and dissolve in 30 ml of *hydrochloric acid* in a stoppered flask. Add 20 ml of *water* and 5 ml of *chloroform* and titrate rapidly with *0.05M potassium iodate* until the chloroform layer is colourless. Each ml of *0.05M potassium iodate* is equivalent to 0.02256 g of $SnCl_2,2H_2O$.

Stannous Chloride Solution

May be prepared by either of the two methods given below:

1. Dissolve 330 g of *stannous chloride* in 100 ml of *hydrochloric acid* and add sufficient *water* to produce 1000 ml.

2. Dilute 60 ml of *hydrochloric acid* with 20 ml of *water*, add 20 g of *tin* and heat gently until gas ceased to be evolved; add sufficient *water* to produce 100 ml, allowing the undissolved tin to remain in the solution.

Starch

Of the Indian Pharmacopoeia.

Starch-Iodine Solution

Dissolve 0.5 g of *soluble starch* in 100 ml of *water* containing 0.5 g of *potassium iodide*.

Starch, Soluble

Starch which has been treated with *hydrochloric acid* until, after being washed, it forms an almost clear limpid solution in hot *water*.

DESCRIPTION – Fine, white powder.

SOLUBILITY – Soluble in hot *water*, usually forming a slightly turbid solution.

ACIDITY OR ALKALINITY – Shake 2 g with 20 ml of *water* for three minutes and filter; the filtrate is not alkaline or more than faintly acid to *litmus paper*.

SENSITIVITY – Mix 1 g with a little cold *water* and add 200 ml of boiling *water*. Add 5 ml of this solution to 100 ml of *water* and add 0.05 ml of *0.1N iodine*. The deep blue colour is discharged by 0.05 ml of *0.1N sodium thiosulphate*.

ASH – Not more than 0.3 per cent, Appendix 3.3.22.

LOSS ON DRYING – Loses not more than 15.0 per cent, when dried to constant weight at 100°, Appendix 5.8.

Starch Substrate

Stir an amount of *soluble starch* equivalent to 1.0 g of the dried substance in a beaker with 5 ml of *water*. Add, stirring continuously, to 75 ml of boiling *water*. Rinse the beaker with two quantities, each 5 ml of *water*, add the washings to the hot starch solution and reboil for two minutes, stirring continuously. Cool to 25°, dissolve 5 g of *sodium chloride* in the starch solution and add sufficient *water* to produce 100 ml. Dilute 10.0 ml of this solution to 100.0 ml with *buffer solution pH 6.0* in the analysis of bacterial amylase and with *acetate buffer pH 5.0* in the analysis of fungal amylase. Each ml of *starch substrate* contains 1.0 mg of dry, soluble starch.

NOTE – Starch Substrate must be freshly prepared.

Stearic Anhydride : $(C_{17}H_{35}CO)_2O$ = 550.96

Contains not less than 98.0 per cent of $C_{36}H_{70}O_3$.

DESCRIPTION – White, waxy, crystalline flakes.

SOLUBILITY – Soluble in *xylene*.

MELTING RANGE – 68° to 72°, Appendix 5.11.

ASSAY – To 4 g add 25 ml of a 4.5 per cent solution of *morpholine* in *methyl alcohol* and heat under a reflux condenser for fifteen minutes. Cool, rinse the condenser with *methyl alcohol*, and add 25 ml of *0.5N hydrochloric acid* with constant stirring. Crush any lumps that may have formed and titrate with *N sodium hydroxide*, using a solution in *methyl alcohol* containing 0.8 per cent of *dimethyl yellow* and 0.08 per cent of *methylene blue* as indicator. Repeat the operation without the sample; the difference between the titrations represents the amount of sodium hydroxide required by the stearic anhydride taken. Each ml of *N sodium hydroxide* is equivalent to 0.5510 g of $C_{36}H_{70}O_3$.

Sucrose

Of the Indian Pharmacopoeia.

Sudan Red G : CI 26100; Sudan III; Solvent Red 23; 1-(4-Phenylazophenylazo)-2-naphthol; $C_{22}H_{16}N_4O$ = 352.40

DESCRIPTION – Reddish-brown powder.

SOLUBILITY – Insoluble in *water*; soluble in *chloroform*, in *glacial acetic acid*; moderately soluble in *alcohol*, in *solvent ether* and in *acetone*.

It complies with the following test :

HOMOGENEITY – Carry out the method for *thin-layer chromatography*, Appendix 5.4.3, using *silica gel G* as the coating substance and *methylene chloride* as the mobile phase. Apply to the chromatoplate 10 µl of a 0.01 per cent w/v solution of the *sudan red G* in *methylene chloride*; the chromatogram shows only one spot.

Sulphamic Acid : $NH_2.SO_3H$ = 97.09

Contains not less than 98.0 per cent of H_3NO_3S.

DESCRIPTION – White crystals or a crystalline powder.

SOLUBILITY – Readily soluble in *water*.

MELTING RANGE – 203° to 205°, with decomposition, Appendix 5.11.

SULPHATE – 0.5 g complies with the *limit test for sulphates*, Appendix 3.2.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Carry out the **ASSAY** described under ammonium sulphamate, using 3 g. Each ml of *N sulphuric acid* is equivalent to 0.09710 g of H_3NO_3S.

Sulphanilamide : $H_2N.C_6H_4.SO_2.NH_2$ = 172.21

Contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of $C_6H_8O_2N_2S$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White or almost white, crystalline powder.

SOLUBILITY – Slightly soluble in *water*; sparingly soluble in *alcohol*; freely soluble in *acetone*; practically insoluble in *chloroform*, in *solvent ether* and in *benzene*.

ACIDITY OR ALKALINITY – A saturated solution in *water* is neutral to *litmus solution*.

MELTING RANGE – 164.5° to 166.5°, Appendix 5.11.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 0.5 per cent of its weight, Appendix 5.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Carry out the **ASSAY** described under Sulphacetamide Sodium. Each ml of *0.1M sodium nitrite* is equivalent to 0.01722 g of $C_6H_8O_2N_2S$.

STORAGE – Sulphanilamide should be kept in a well-closed container, protected from light.

Sulphanilic Acid : $H_2N.C_6H_4.SO_3H = 173.19$

Contains not less than 99.0 per cent of $C_6H_7NO_3S$.

DESCRIPTION – White or nearly white crystals or powder.

SOLUBILITY – Soluble in 33 parts of hot *water*, giving a clear and colourless solution, which deposits crystals on cooling.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately 5 g, suspend in 100 ml of *water* and titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1732 g of $C_6H_7NO_3S$.

Sulphanilic Acid, Diazotised

Dissolve 0.2 g of *sulphanilic acid* in 20 ml of *N hydrochloric acid*, cool in ice, add sufficient of a 10 per cent w/v solution of *sodium nitrite* in *water* until an immediate blue colour is given with *starch-iodide paper*, and add 0.05 g of *sulphamic acid*.

Diazotised Sulphanilic Acid should be freshly prepared.

Sulphaphenazole

Of the Indian Pharmacopoeia.

Sulphathiazole : $C_9H_9N_3O_2S_2 = 255.32$

Contains not less than 99.0 per cent of $C_9H_9N_3O_2S_2$, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – White or yellowish-white crystals or powder; odourless or almost odourless.

SOLUBILITY – Almost insoluble in *water*, slightly soluble in *alcohol*, in dilute mineral acids and in aqueous solutions of alkali hydroxides and carbonates.

MELTING RANGE – 200° to 203°, Appendix 5.11.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 0.5 per cent of its weight, Appendix 5.8.

ASSAY – Carry out the **ASSAY** described under Sulphacetamide Sodium. Each ml of *0.1M sodium nitrite* is equivalent to 0.02553 g of $C_9H_9N_3O_2S_2$.

4-Sulphamoyl Benzoic Acid : *p*-Carboxybenzene Sulphonamide; $HO_2C.C_6H_4.SO_2.NH_2 = 201.20$

MELTING POINT – About 291°, Appendix 5.11.

Sulphur Dioxide : $SO_2 = 64.06$

DESCRIPTION – A colourless gas; odour, characteristic and unpleasant; the compressed substance is a colourless liquid.

SOLUBILITY – When passed into *water*, yields a clear colourless solution which is strongly acid to *litmus paper*.

Sulphur, Precipitated : S = 32.06

DESCRIPTION – Pale greyish-yellow or pale greenish-yellow, soft powder, free from grittiness; odourless; tasteless. Burns with a blue flame with the production of sulphur dioxide.

SOLUBILITY – Almost insoluble in *water*, and in *alcohol*, completely soluble in *carbon disulphide*.

MICROSCOPICAL APPEARANCE – Under a microscope it consists of grouped amorphous subglobular particle free from crystals.

ACIDITY – Thoroughly agitate 5 g with 50 ml of *carbon dioxide-free water* and titrate with *0.1N sodium hydroxide*, using *phenolphthalein solution* as indicator; not more than 0.5 ml is required.

MATTER INSOLUBLE IN CARBON DISULPHIDE – Shake 1.0 g with 20 ml of *carbon disulphide*, allow to stand for ten minutes, filter, wash the residue with *carbon disulphide*, and dry; the residue weighs not more than 5 mg.

RESIDUE ON IGNITION – Leaves not more than 0.25 per cent w/w of the residue, when ignited to constant weight.

WATER – Not more than 0.5 per cent w/v, Appendix 3.3.25, using *pyridine* instead of *methyl alcohol*.

Sulphuric Acid : $H_2SO_4 = 98.07$

Contains not less than 97.0 per cent w/w of H_2SO_4. About 36 *N* in strength.

DESCRIPTION – Colourless, corrosive liquid of oily consistence, evolves much heat when added to *water*.

WT. PER ML – About 1.84 g, Appendix 5.19.

ARSENIC – Not more than 4 parts per million, Appendix 3.2.1.

IRON – 1 ml complies with the *limit test for iron*, Appendix 3.2.5.

HEAVY METALS – Not more than 20 parts per million, determined by the following methods:

Add 1.1 ml of the acid to about 10 mg of *sodium carbonate* dissolved in a few ml of *water*, and carefully heat over a free flame until nearly dry. Add 1 ml of *nitric acid*, evaporate to dryness on a water-bath. Take up the residue in 20 ml of *water*, and neutralise to *phenolphthalein solution* with *0.1N sodium hydroxide*. Add 1 ml of *dilute acetic acid* and dilute to 40 ml. Prepare a control to contain 0.040 mg of Pb and 1 ml of *dilute acetic acid* in a volume of 40 ml. Add to each solution 10 ml *hydrogen sulphide solution*. The colour of the solution of the sample is not darker than that of the control solution.

CHLORIDE – 5 ml diluted with *water* and neutralised with *dilute ammonia solution*, complies with the *limit test for chlorides*, Appendix 3.2.2.

NITRATE – Add 5 ml to a mixture of 5 ml of *water* and 0.5 ml of *indigo carmine solution*; the colour is not discharged within one minute.

OXIDISABLE IMPURITIES – Add 15 ml to a mixture of 60 ml of *water* and 0.05 ml of *0.1N potassium permanganate*; the pink colour is not discharged within five minutes.

RESIDUE ON IGNITION – Leaves not more than 0.01 per cent w/v of the residue, when ignited to constant weight.

ASSAY – Weigh accurately about 2 g, mix with about 40 ml of *water*, cool, and titrate with *N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.04904 g of H_2SO_4.

Sulphuric Acid, *x*N

Solutions of any normality *x*N may be prepared by diluting 54*x* ml of *sulphuric acid* to 1000 ml with *water*.

Sulphuric Acid (80 per cent w/v)

Mix four volumes of *sulphuric acid* and one volume of *water*, and cool.

Sulphuric Acid (50 per cent v/v)

Mix equal volumes of *sulphuric acid* and *water*, and cool.

Sulphuric Acid (50 per cent w/w)

Mix equal weights of *sulphuric acid* and *water*, and cool.

Sulphuric Acid (25 per cent v/v)

Mix one volume of *sulphuric acid* and three volumes of *water*, and cool.

Sulphuric Acid (14 per cent v/v)

Mix 14.0 ml of *sulphuric acid* and 86.0 ml of *water*, and cool.

Sulphuric Acid, Dilute

Contains approximately 10 per cent w/w of H_2SO_4.

Dilute 57 ml of *sulphuric acid* to 1000 ml with *water*.

Sulphuric Acid in Methanol

Add cautiously *sulphuric acid* to 30 ml of chilled *dehydrated methyl alcohol* in 100-ml volumetric flask, in small quantities and with mixing. Adjust to room temperature and dilute to volume with sulphuric acid. Mix well.

Sulphuric Acid, Nitrogen-free

Sulphuric acid which contains not less than 98.0 per cent w/w of H_2SO_4, and complies with the following additional test:

NITRATE – Mix 45 ml with 5 ml of *water*, cool and add 8 mg of *diphenylbenzidine*; the solution is colourless or not more than very pale blue.

Tannic Acid : Tannin; $C_{76}H_{52}O_{46}$ = 1701.24

DESCRIPTION – Yellowish-white or light brownish glistening scales, light masses or an impalpable powder; odour, characteristic; taste, strongly astringent.

SOLUBILITY – Very soluble in *water* in *acetone* and in *alcohol*; practically insoluble in *solvent ether* and in *chloroform*; slowly soluble in *glycerin*.

GUMS, DEXTRIN, SUGARS, AND SALTS – To 2 ml of a 20 per cent w/v solution add 2 ml of *alcohol (90 per cent)*; the solution remains clear and shows no turbidity on the addition of 1 ml of *solvent ether*.

LOSS ON DRYING – Loses not more than 9.0 per cent of its weight, when dried to constant weight at 105°, Appendix 5.8.

SULPHATED ASH – Not more than 0.2 per cent, Appendix 3.2.7.

Tannic Acid Solution

A 10.0 per cent w/v solution of *tannic acid* in *water*.

NOTE – Aqueous solutions of tannic acid decompose on keeping.

Tartaric Acid

Of the Indian Pharmacopoeia.

Tetrabutylammonium Iodide : $[CH_3(CH_2)_3]_4NI$ = 369.37

Contains not less than 98 per cent of $C_{16}H_{36}NI$.

DESCRIPTION – White or slightly coloured crystals or crystalline powder.

SOLUBILITY – Soluble in *alcohol* and in *solvent ether*; slightly soluble in *water*.

MELTING RANGE – Between 146° to 147°, Appendix 5.11.

SULPHATED ASH – Not more than 0.02 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1.2 g and dissolve in 30 ml of *water*. Add 50 ml of *0.1N silver nitrate* and 5 ml of *dilute nitric acid*. Titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.03694 g of $C_{16}H_{36}NI$.

Tetracycline

Of the Indian Pharmacopoeia.

***n*-Tetradecane :** $CH_3(CH_2)_{12}CH_3$ = 198.39

DESCRIPTION – A clear, colourless liquid.

SOLUBILITY – Miscible with *alcohol*.

REFRACTIVE INDEX – At 20°, 1.428 to 1.429, Appendix 5.14.

WT. PER ML – At 20°, about 0.76 g, Appendix 5.19.

Tetrahydrofuran : $CH_2(CH_2)_2CH_2O$ = 72.11

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Miscible with *water*, with *alcohol* and with *solvent ether*.

WT. PER ML – At 20°, 0.885 to 0.888 g, Appendix 5.19.

PEROXIDE – Place 10 ml of *ferrous thiocyanate reagent* in a 30 ml stoppered-bottle previously filled with *carbon dioxide*. Completely fill the bottle with the sample, insert the stopper, shake vigorously and allow to stand in the dark for five minutes; no pink colour is produced.

DISTILLATION RANGE – Not less than 95 per cent distils between 64° and 66°, Appendix 5.3.

NOTE – It is dangerous to determine the Distillation Range on a sample that does not comply with the test for peroxide.

Tetramethylammonium Chloride : $(CH_3)_4NCl$ = 109.60

Contains not less than 98.0 per cent of $C_4H_{12}NCl$.

DESCRIPTION – Colourless crystals.

SOLUBILITY – Soluble in *water* and in *alcohol*; insoluble in *chloroform*.

ASSAY – Weigh accurately about 0.2 g, dissolve in 50 ml of *water* and 10 ml of *dilute nitric acid*, add 50.0 ml of *0.1N silver nitrate*, and mix, Add 2 ml of *ferric ammonium sulphate solution* and 5 ml of *nitrobenzene*, and titrate with shaking the excess silver nitrate with *0.1N ammonium thiocyante*. Each ml of *0.1N silver nitrate* is equivalent to 0.010969 g of $C_4H_{12}NCl$.

Tetramethylammonium Hydroxide Solution (25 per cent)

Contains 25.0 per cent w/w of $C_4H_{13}NO$ (limits, 23.75 to 26.25)

DESCRIPTION – Clear, colourless or very pale yellow liquid having a strong ammonia-like odour.

SOLUBILITY – Miscible with *water* and with *alcohol*.

RESIDUE ON EVAPORATION – Evaporate 5 ml on a water bath, and dry at 105° for one hour; the weight of the residue is equivalent to not more than 0.02 per cent of the weight of the sample.

AMMONIA AND OTHER AMINES – Weigh accurately a quantity of the solution, corresponding to about 0.3 g of $C_4H_{13}NO$ in a weighing bottle tared with 5 ml of *water*. Add 4 ml of *N hydrochloric acid*, evaporate on a water-bath to dryness, and dry at 105° to constant weight; the weight of the residue multiplied by 0.8317, represents the quantity, in mg, of $(CH_3)_4NOH$ in the portion of sample taken and corresponds to within ± 0.2 per cent of the quantity of $C_4H_{13}NO$ found in the **ASSAY**.

ASSAY – To about 1.5 g, accurately weighed in a glass-stoppered flask, add 50 ml of *water* and titrate with *0.1N hydrochloric acid* using *methyl red solution* as indicator. Each ml of *0.1N hydrochloric acid* is equivalent to 0.009115 g of $C_4H_{13}NO$.

Tetramethylammonium Hydroxide Solution (10 per cent)

The solution is commercially available as such or may be prepared by diluting a suitable amount of *tetramethylammonium hydroxide solution (25 per cent)* with *water*. Contains 10.0 per cent w/w of $C_4H_{13}NO$ (limits, 9.5 to 10.5).

DESCRIPTION, SOLUBILITY, RESIDUE ON EVAPORATION, AMMONIA AND OTHER AMINES – Complies with the requirements described under tetramethylammonium hydroxide solution (25 per cent).

ASSAY – Carry out the **ASSAY** described under Tetramethylammonium Hydroxide Solution (25 per cent).

NNN'N'-Tetramethyl-*p*-phenylenediammonium Dichloride : $C_6H_4[N(CH_3)_2]_2.2HCl = 237.17$

DESCRIPTION – White or slightly tinted, crystalline powder.

SOLUBILITY – Soluble in *water*.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

Thiamine Hydrochloride

Of the Indian Pharmacopoeia.

Thioacetamide : $CH_3.CS.NH_2 = 75.1$

Contains not less than 98.0 per cent of C_2H_5NS.

DESCRIPTION – White crystals or a crystalline powder.

SOLUBILITY – Soluble in *water* and in *alcohol*; insoluble in *benzene*.

MELTING RANGE – 112° to 115°, Appendix 5.11.

SULPHATED ASH – Not more than 0.05 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 0.15 g and transfer to conical flask, add 50 ml of *0.1N silver nitrate*, stopper the flask, and allow to stand for thirty minutes with frequent shaking. Add 15 ml of *dilute nitric acid*, mix, and filter through a small plug of cotton wool. Wash the flask and residue with 50 ml of *water*, add the washings to the filtrate, and titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.003757 g of C_2H_5NS.

Thioacetamide Reagent

Add 1 ml of a mixture of 15 ml of *N sodium hydroxide*, 5 ml of *water*, and 20 ml of *glycerin* to 0.2 ml of a 4 per cent w/v solution of *thioacetamide* in *water*. Heat on a water-bath for twenty seconds and cool.

Thioacetamide Reagent should be prepared immediately before use.

Thioglycollic Acid : Mercaptoacetic Acid
$HS.CH_2.COOH = 92.11$

Contains not less than 89.0 per cent w/w of $C_2H_4O_2S$, as determined by both parts of the **ASSAY** described below:

DESCRIPTION – Colourless or nearly colourless liquid; odour strong and unpleasant.

IRON – Mix 0.1 ml with 50 ml of *water*, and render alkaline with *strong ammonia solution*; no pink colour is produced.

ASSAY – (1) Weigh accurately about 0.4 g and dissolve in 20 ml of *water* and titrate with *0.1N sodium hydroxide* using *cresol red solution* as indicator. Each ml of *0.1N sodium hydroxide* is equivalent to 0.009212 g of $C_2H_4O_2S$.

(2) To the above neutralised solution add 2 g of *sodium bicarbonate* and titrate with *0.1N iodine*. Each ml of *0.1N iodine* is equivalent to 0.009212 g of $C_2H_4O_2S$.

Thiomersal

Of the Indian Pharmacopoeia.

Thiourea : $H_2N.CS.NH_2 = 76.12$

Contains not less than 99.0 per cent of CH_4N_2S, calculated with reference to the substance dried to constant weight at 105°.

DESCRIPTION – Colourless crystalline powder, having a faint characteristic odour.

SOLUBILITY – Soluble in *water*; slightly soluble in *alcohol*.

MELTING RANGE – 176° to 180°, Appendix 5.11.

LOSS ON DRYING – When dried to constant weight at 105°, loses not more than 1.0 per cent of its weight, Appendix 5.8.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 1 g, dissolve in *water* and dilute to 250 ml; to 20 ml of this solution add 25 ml of *0.1N silver nitrate* and 10 ml of *dilute ammonia solution*, shake vigorously for two minutes, heat to boiling, cool, add 60 ml of *dilute nitric acid*, shake, and filter; wash the precipitate with *water* and titrate the filtrate and washings with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.003806 g of CH_4N_2S.

Thoron

The sodium salt of 4-(O-arsonophenylazo)-3-hydroxy-naphthalene-2, 7-disulphonic acid.

Thrombokinase Extract

Extract 1.5 g of *acetone-dried ox brain* with 60 ml of *water* for ten to fifteen minutes at 50°, centrifuge for two minutes at 1500 rpm, and decant the supernatant liquid. Cresol, 0.3 per cent, may be added as a bactericide. Store in a refrigerator. Under these conditions the extract will retain its activity for several days.

Thymol

Of the Indian Pharmacopoeia.

Tin : Sn = 118.69

DESCRIPTION – Silver-white sticks or granules.

LEAD – Digest 10 g with 40 ml of *nitric acid* on a water-bath; evaporate to dryness. Warm the residue with 5 ml of *nitric acid* and 50 ml of *water*, filter and wash with *water*. To the filtrate add 2 ml of *sulphuric acid* and evaporate; cool, add 5 ml of *alcohol*, filter, wash with *alcohol* and ignite. The residue weighs not more than 0.1 g.

Titanium Dioxide

Of the Indian Pharmacopoeia.

Titanium Dioxide Solution

To 0.1 g of *titanium dioxide* add 100 ml of *sulphuric acid* and heat cautiously with occasional stirring, until solution is effected and fumes are evolved; cool and store in a glass-stoppered bottle.

Titanous Chloride Solution

A 15 per cent w/v solution of *titanous chloride* in *dilute hydrochloric acid*.

DESCRIPTION – Dull purplish-brown liquid with a strongly acid reaction.

CONTENT OF $TiCl_3$ – When diluted and titrated according to the directions given under *0.1N titanous chloride*, Appendix 7.5, the strength of the diluted solution is not less than 0.1 *N*.

STORAGE – Titanous Chloride Solution must be protected from the atmosphere to avoid oxidation.

Toluene : $C_6H_5.CH_3 = 92.14$

DESCRIPTION – Clear, colourless, flammable liquid.

SOLUBILITY – Insoluble in *water*, miscible with *ethyl alcohol*.

DISTILLATION RANGE – Not less than 95.0 per cent distil between 109° and 111°, Appendix 5.3.

WT. PER ML – At 20°, 0.860 to 0.865 g, Appendix 5.19.

SULPHUR COMPOUNDS – Boil 10 ml with 1 ml of *ethyl alcohol* and 3 ml of *potassium plumbite solution* for fifteen minutes under reflux condenser, and set aside for five minutes; the aqueous layer remains colourless.

REACTION WITH SULPHURIC ACID – Shake 5 ml with 5 ml of *sulphuric acid*; the toluene remains colourless and the acid does not become more than pale yellow.

NON-VOLATILE MATTER – Leaves not more than 0.01 per cent w/v of residue, when evaporated on a water-bath and dried to constant weight at 105°.

Toluene, Anhydrous

Toluene, which has been dried over *anhydrous sodium sulphate*.

Triacetin : Glycerol Triacetate
$CH_2(CO_2{\cdot}CH_3){\cdot}CH(CO_2{\cdot}CH_3){\cdot}CH_2(CO_2{\cdot}CH_3) = 218.20$.

Contains not less than 95.0 per cent and not more than 99.0 per cent w/w of $C_9H_{14}O_6$.

DESCRIPTION – Clear, colourless or pale straw-coloured liquid.

ACID VALUE – Not more than 0.3, Appendix 3.3.15.

WT. PER ML – At 25°, 1.154 to 1.158 g, Appendix 5.19.

ASSAY – Carry out the method for *determination of esters*, Appendix 3.3.2. Each ml of *0.5N alcoholic potassium hydroxide* is equivalent to 0.03637 g of $C_9H_{14}O_6$.

Tributyl Phosphate : Tributyl Orthophosphate
$[CH_3{\cdot}(CH_2)_3]_3\ PO_4 = 266.32$.

DESCRIPTION – Clear, colourless liquid.

SOLUBILITY – Slightly soluble in *water*; miscible with *ethyl alcohol* and with *solvent ether*.

REFRACTIVE INDEX – At 20°, 1.424 to 1.425, Appendix 5.14.

WT. PER ML – At 20°, 0.974 to 0.977 g, Appendix 5.19.

NOTE – Tributyl phosphate should be washed three times before use with one-sixth of its volume of a solution containing 10 per cent w/v of sodium chloride and 1.3 per cent w/v of sodium dihydrogen phosphate.

Trichloroacetic Acid : $CCl_3.COOH = 163.40$

Contains not less than 98.0 per cent of $C_2HCl_3O_2$.

DESCRIPTION – Colourless, very deliquescent crystals or crystalline masses; odour, slight or pungent and characteristic.

SOLUBILITY – Very soluble in *water*, in *alcohol*, and in *solvent ether*.

MELTING RANGE – 55° to 57°, Appendix 5.11.

CHLORIDE – 1 g complies with the *limit test for chlorides*, Appendix 3.2.2.

NITRATE – To a solution of 1 g in 10 ml of *water*, add 0.5 ml of *indigo carmine solution* and 10 ml of *nitrogen-free sulphuric acid*, and heat to boiling; the solution remains blue.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

ASSAY – Weigh accurately about 5 g and dissolve in 50 ml of *water*. Titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1634 g of $C_2HCl_3O_2$.

Triethanolamine : $N(CH_2.CH_2OH)_3 = 149.19$

DESCRIPTION – Colourless liquid; odour, ammoniacal.

SOLUBILITY – Miscible with *water* and with *ethyl alcohol* slightly soluble in *solvent ether*.

REFRACTIVE INDEX – At 20°, 1.482 to 1.485, Appendix 5.14.

WT. PER ML – About 1.13 g, Appendix 5.19.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

Trimethylchlorosilane : Chlorotrimethylsilane $(CH_3)_3.SiCl = 108.65$

DESCRIPTION – Clear, colourless liquid.

REFRACTIVE INDEX – At 20°, 1.459 to 1.461, Appendix 5.14.

WT. PER ML – About 0.86 g, Appendix 5.19.

2,3,5-Triphenyl Tetrazolium Chloride : Tetrazolium salt; $[C_6H_5{\cdot}CNN{\cdot}(C_6H_5){\cdot}N^+ \ (C_6H_5){:}N]Cl^- = 334.81$

Contains not less than 95.0 per cent of $C_{19}H_{15}N_4Cl$.

DESCRIPTION – Pale-yellow or cream-coloured powder.

SOLUBILITY – Soluble in *water*.

MELTING RANGE – 239° to 241°, with decomposition, Appendix 5.11.

ASSAY – Weigh accurately about 1 g and dissolve in a mixture of 45 ml of *water* and 5 ml of *dilute nitric acid*. Add 50 ml of *0.1N silver nitrate* and heat to boiling; cool, add 3 ml of *nitrobenzene*, shake vigorously, and titrate the excess of silver nitrate with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.03348 g of $C_{19}H_{15}N_4Cl$.

Triphenyltetrazolium Chloride Solution

A 0.5 per cent w/v solution of *2,3,5-triphenyltetrazolium chloride* in *aldehyde-free ethyl alcohol*.

Triphenyltetrazolium Chloride Solution should be freshly prepared.

Tropaeolin O : CI 14270; Resorcin Yellow; Chrysoin S; Sulpho Orange

DESCRIPTION – A yellowish-orange or brown, fine powder.

Complies with the following test:

HOMOGENEITY – Carry out the test for **SEPARATING POWER** described under *Cellulose MN 300*, applying to the plate a solution containing only the tropaeolin O; the chromatogram shows only one spot at an R_f of about 0.9.

Gives a yellow solution in moderately alkaline solutions, and an orange colour in strongly alkaline solutions (pH range, 11.0 to 12.7).

Trypsin

Freeze-dried, salt-free beef trypsin containing not less than 9000 benzoylarginine ethyl ester Units per mg.

Trypsin should be stored at a temperature not exceeding 4°.

L-Tryptophane : $C_{11}H_{12}N_2O_2 = 204.23$

DESCRIPTION – White or not more than slightly yellow powder or leaflets.

SOLUBILITY – Slightly soluble in *water* and in *alcohol*; soluble in dilute acids and in solutions of alkali hydroxides.

SPECIFIC OPTICAL ROTATION – Between −30.0° and −33.0°, determined in a 1.0 per cent w/v solution of the material, dried at 105° for three hours, Appendix 5.12.

TYROSINE – Dissolve 0.1 g in 3 ml of *dilute sulphuric acid*, add 10 ml *mercuric sulphate solution* and heat on a water-bath for ten minutes. Filter, wash with 5 ml of *mercuric sulphate solution* and add to the combined filtrate 0.5 ml of a 5.0 per cent w/v solution of *sodium nitrite*; no red colour is produced within fifteen minutes.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 0.3 per cent, determined by drying in an oven at 105° for three hours, Appendix 5.8.

Turmeric

Turmeric consists of the dried and fresh rhizomes of *curcumalonga* Linn. (Fam. Zingiberaceae). It contains not less than 4.0 per cent of volatile oil.

DESCRIPTION – *Macroscopical* – The rhizomes are ovate, oblong or pyriform (round turmeric) or cylindrical and often short-branched (long turmeric). The round form is about half as broad as long, the long form from 2 to 5 cm long and about 1 to 1.8 cm thick; externally yellowish to yellowish-brown, with root scars and annulations, the latter from the scars of leaf bases. The internal colour varies from yellow to yellow-orange to orange; waxy, Central cylinder twice as broad as the cortex, Fracture, horny.

Microscopical – The transverse section of the rhizome is characterised by the presence of mostly thin walled rounded parenchyma cells, scattered vascular bundles, definite endodermis and a few layers of cork developed under the epidermis and scattered oleoresin cells with brownish contents. The epidermis is thick-walled,

consisting of cells, cubical in shape, of various dimensions. The cork cambium is developed from the sub-epidermal layers and even after the development of the cork, the epidermis is retained. Cork is generally composed of 4 to 6 layers of thin-walled brick-shaped parenchymatous cells. The parenchyma of the pith and cortex contains grains altered to a paste, in which sometimes long lens shaped unaltered starch grains of 4 to 15 μ diameter are found. Oil cells have suberised walls and contain either orange-yellow globules of a volatile oil or amorphous resinous masses. Cortical vascular bundles are scattered and are of a collateral type. The vascular bundles in the pith region are mostly scattered and they form discontinuous ring just under the endodermis. The vessels have mainly spiral thickenings and only a few have reticulate and annular structure.

BEHAVIOUR WITH ACIDS AND ALKALIS – (A) On the addition of *sulphuric acid* or a mixture of *sulphuric acid* and *alcohol* a deep crimson colour is produced.

(B) On the addition of *boric acid*, a reddish-brown colour is formed which, on the addition of alkalis, changes to greenish-blue.

(C) A piece of filter paper impregnated with an alcoholic extract, dried, and then moistened with a solution of *boric acid* slightly acidified with *hydrochloric acid*, and again dried, assumes a pink or brownish-red colour which becomes deep blue or greenish-black on the addition of alkali.

FOREIGN ORGANIC MATTER – Not more than 2 per cent, Appendix 3.3.22.

ASH – Not more than 9 per cent, Appendix 3.3.22.

ALCOHOL SOLUBLE EXTRACTIVE – Not less than 8 per cent, Appendix 3.3.22.

Tyrosine : L-Tyrosine; $HO.C_6H_4.CH_2.CH(NH_2)CO_2H$ = 181.19

Contains not less than 98.5 per cent of $C_9H_{11}NO_3$.

DESCRIPTION – White powder.

SOLUBILITY – Slightly soluble in *water*; more soluble in dilute solutions of *sodium hydroxide* and in *dilute hydrochloric acid*.

SULPHATED ASH – Not more than 0.1 per cent, Appendix 3.2.7.

LOSS ON DRYING – Loses not more than 0.5 per cent of its weight, when dried to constant weight at 100°, Appendix 5.8.

ASSAY – Carry out the method for *determination of nitrogen*, Appendix 3.3.5, using about 0.5 g, accurately weighed and 9.0 ml of *nitrogen-free sulphuric acid*. Each ml of *0.1N sulphuric acid* is equivalent to 0.01812 g of $C_9H_{11}NO_3$.

Uracil : $C_4H_4N_2O_2$ = 112.1

DESCRIPTION – A white to cream-coloured, crystalline powder.

SOLUBILITY – Slightly soluble in *water*; less soluble in *alcohol*; soluble in *dilute ammonia solution* and in *sodium hydroxide solution*.

MELTING TEMPERATURE – Above 300°, Appendix 5.11.

SULPHATED ASH – Not more than 0.01 per cent, Appendix 3.2.7.

LOSS ON DRYING – Not more than 2.0 per cent, determined by drying in an oven at 105° for two hours, Appendix 5.8.

Uranyl Acetate : $UO_2(CH_3COO)_2,2H_2O$ = 424.15

DESCRIPTION – Bright yellow, crystalline powder.

SOLUBILITY – Slowly soluble in *water* acidified with *acetic acid*.

ALKALIS – Dissolve 2 g in 50 ml of *water*, heat to boiling and add *dilute ammonia solution* until precipitation is complete; filter, evaporate the filtrate to dryness, moisten with *sulphuric acid* and ignite gently to constant weight; the residue weighs not more than 6 mg.

Uranyl Zinc Acetate Solution

Dissolve 10 g of *uranyl acetate* by heating in 50 ml of *water* and 5 ml of *acetic acid*; dissolve 30 g of *zinc acetate* by heating with 30 ml of *water* and 3 ml of *acetic acid*; mix the two solutions, allow to cool and filter.

Urea

Of the Indian Pharmacopoeia.

Vanadyl Sulphate : $VOSO_4,xH_2O$ = 163.00 (anhydrous)

Contains not less than 97.0 per cent of $VOSO_4$.

DESCRIPTION – Blue, hygroscopic crystals.

SOLUBILITY – Slowly and usually incompletely soluble in *water*.

LOSS ON DRYING – 1 g when dried to constant weight at 220°, loses not more than 50 per cent of its weight, Appendix 5.8.

PENTAVALENT VANADIUM – Not more than 0.5 per cent, calculated on the dried basis, when determined by the following method:

Heat 1 g, accurately weighed, with 50 ml of *water* and 5 ml of *hydrochloric acid* in a stoppered flask until dissolved, cool, add 2 g of *potassium iodide*, insert the stopper, and allow it to stand for 30 minutes. Add 50 ml of *water* and titrate the liberated iodine with *0.1N sodium*

thiosulphate, using *starch solution* as the indicator. Correct for the volume of sodium thiosulphate consumed by a blank. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.005096 g of pentavalent vanadium.

SUBSTANCES NOT PRECIPITATED BY AMMONIA – Dissolve 1 g by heating with 20 ml of *water* and 2 ml of *hydrochloric acid*. Dilute with *water* to about 75 ml and neutralize to *litmus paper* with *dilute ammonia solution*. Transfer the solution to a cylinder, add 5 ml of *strong ammonia* and sufficient *water* to make 100 ml and allow to stand overnight. Decant 50 ml of the supernatant liquid through a filter, add 5 drops of *sulphuric acid*, evaporate to dryness, and *ignite*; the residue weighs not more than 10 mg.

Assay : Weigh accurately about 0.4 g of the dried sample obtained in the test for **LOSS ON DRYING**, and transfer with 15 to 20 ml of *water* into a beaker. Add 3 ml of *sulphuric acid*, cover the beaker with watch glass, and heat on a water-bath until all dissolves, cool, dilute with 125 ml of *water*, and titrate with *0.1N potassium permanganate* to the production of a pinkish colour that persists for 1 minute. Each ml of *0.1N potassium permanganate* is equivalent to 0.01630 g of $VOSO_4$.

Vanadyl Sulphate Solution

Dissolve 0.1 g of vanadyl sulphate in 20 ml of *sulphuric acid* in a 200 ml volumetric flask, cautiously add *glacial acetic acid* to volume and mix.

Vanillin

Of the Indian Pharmacopoeia.

Water

Purified Water of the Indian Pharmacopoeia.

Water, Ammonia-free

Water which complies with the following additional test:

To 50 ml, add 2 ml of *alkaline potassium mercuri-iodide solution*; no colour is produced.

Water, Carbon Dioxide-free

Water which has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

Water for Injection

Of the Indian Pharmacopoeia.

Water for Injection, Sterile

Of the Indian Pharmacopoeia.

Water, Purified

Of the Indian Pharmacopoeia.

Xanthydrol : Xanthen-2-ol; $C_{13}H_{10}O_2$ = 198.21

Contains not less than 98.0 per cent of $C_{13}H_{10}O_2$.

DESCRIPTION – White to pale yellow powder.

SOLUBILITY – Almost insoluble in *water*; soluble in *alcohol*, in *chloroform*, in *solvent ether*, and in *glacial acetic acid*.

MELTING RANGE – 121° to 124°, Appendix 5.11.

SULPHATED ASH – Not more than 2.0 per cent, Appendix 3.2.7.

ASSAY – Weigh 0.1 g into a 250-ml beaker and place in an ice-bath. Add, with stirring, 50 ml of *sulphuric acid* in small portions and 50 ml of *0.1N potassium dichromate*. Boil for five minutes, cool, and dilute to 500 ml with *water*. To 50 ml of this solution add 2 g of *potassium iodide* and titrate the liberated iodine with *0.1N sodium thiosulphate*, using *starch solution*, added towards the end of the titration, as indicator. Each ml of *0.1N potassium dichromate* is equivalent to 0.0003416 g of $C_{13}H_{10}O_2$.

Xanthydrol Reagent

Dissolve about 125 mg of *xanthydrol* in 100 ml of *glacial acetic acid*. Add 1 ml of *hydrochloric acid* immediately prior to use.

Xylene : $C_6H_4(CH_3)_2$ = 106.17

DESCRIPTION – Clear, colourless, flammable liquid, consisting mainly of *m*-xylene with smaller proportions of *o*- and *p*-xylenes.

SOLUBILITY – Insoluble in *water*; miscible with *ethyl alcohol*.

DISTILLATION RANGE – Not less than 90.0 per cent distils between 136° and 140°, Appendix 5.3.

WT. PER ML – At 20°, 0.850 to 0.860 g, Appendix 5.19.

SULPHUR COMPOUNDS – Boil 10 ml with 1 ml of *ethyl alcohol* and 3 ml of *potassium plumbite solution* for fifteen minutes under a reflux condenser, and set aside for five minutes; the aqueous layer remains colourless.

REACTION WITH SULPHURIC ACID – Shake 5 ml with 5 ml of *sulphuric acid*; the xylene remains colourless and the acid may become yellow but not brown.

NON-VOLATILE MATTER – Leaves not more than 0.01 per cent w/v of residue, when evaporated on a water-bath, and dried to constant weight at 105°.

Zinc Acetate : $(CH_3.CO_2)_2Zn,2H_2O$ = 219.50

DESCRIPTION – Colourless crystals.

SOLUBILITY – Readily soluble in *water*, forming a clear, colourless solution.

ALKALIS – Dissolve 2 g in 50 ml of *water*; add 1 ml of *glacial acetic acid*, make alkaline with *dilute ammonia solution* and pass in *hydrogen sulphide*. Filter, evaporate the filtrate to dryness, moisten with *sulphuric acid* and ignite gently; the residue weighs not more than 6 mg.

Zinc Chloride

Of the Indian Pharmacopoeia.

Zinc Chloride Solution, Iodinated

Dissolve 20 g of *zinc chloride* and 6.5 g of *potassium iodide* in 10.5 ml of *water*. Add 0.5 g of *iodine* and shake for fiteen minutes; filter, if necessary.

Zinc, Granulated : Zn = 65.38

DESCRIPTION – Bright silver-grey, metallic granules.

IRON – Dissolve 0.5 g in 3 ml of *dilute hydrochloric acid*, add 8 ml of *water* and one drop of *0.1N potassium permanganate*, mix, add 5 ml of 57.0 per cent w/v solution of *ammonium thiocyanate in water* and 10 ml of a mixture of equal volumes of *amyl alcohol* and *amyl acetate*, shake vigorously and allow to separate; any colour produced in the upper layer is not greater than that produced by treating 0.5 ml of *standard iron solution* in the same manner.

ACID-INSOLUBLE MATTER – Dissolve 5 g in a mixture of 20 ml of *hydrochloric acid* and 20 ml of *water*; the solution is clear, colourless, and free from more than traces of insoluble matter.

OXIDISABLE IMPURITIES – Dissolve 10 g in a mixture of 50 ml of *water* and 15 ml of *sulphuric acid* in a vessel from which air is excluded, and add 0.1 ml of *0.1N potassium permanganate*; the pink colour is not discharged within five minutes.

Zinc Oxide

Of the Indian Pharmacopoeia.

Zinc Powder : Zn = 65.38

DESCRIPTION – Dense bluish-grey powder.

SOLUBILITY – Almost completely soluble in *dilute hydrochloric acid* with the evolution of hydrogen.

ASSAY – Weigh accurately about 1 g and shake with 25 g of *ferric ammonium sulphate* and 100 ml of *water* until dissolved, add 100 ml of *dilute sulphuric acid*, dilute with *water* to 500 ml, and titrate 50 ml of this solution with *0.1N potassium permanganate*. Each ml of *0.1N potassium permanganate* is equivalent to 0.003269 g of Zn.

Zinc Reducing Mixture

Mix thoroughly 100 g of *barium sulphate*, 1.0 g of *zinc powder* and 10 g of *manganese sulphate*.

Zinc Sulphate

Of the Indian Pharmacopoeia.

Zinc Sulphate Solution, Dilute

A 0.011 per cent w/v solution of *zinc sulphate* in *water*.

Dilute Zinc Sulphate Solution contains 0.025 mg of Zn in 1 ml.

Zirconyl Alizarin Solution, Acid

Dissolve 0 3 g of *zirconyl chloride* in 50 ml of *water*, add slowly, while rotating the flask, a solution of 70 mg of *alizarin red S* in 50 ml of *water*, and dilute the clear solution to 1000 ml with a solution prepared as follows:

Add 112 ml of *hydrochloric acid* to 500 ml of *water*; to 400 ml of *water* add 37 ml of *sulphuric acid*, make up to 500 ml with *water*, and allow to cool; mix the two solutions.

Acid Zirconyl Alizarin Solution should be prepared at least one hour before use.

Zirconyl Chloride : $ZrOCl_2.8H_2O$ = 322.30

Contains not less than 96 per cent of $ZrOCl_2,8H_2O$.

DESCRIPTION – White or creamy-white, crystalline powder.

SOLUBILITY – Soluble in *water*.

ASSAY – Dissolve 0.6 g in 50 ml of *water* containing 5 ml of *nitric acid*. Add 50.0 ml of *0.1N silver nitrate* and 3 ml of *dibutylphthalate*, shake well, and titrate the excess of *0.1N silver nitrate* with *0.1N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of *0.1N silver nitrate* is equivalent to 0.01611 g of $ZrOCl_2,8H_2O$.

7.5 VOLUMETRIC REAGENTS AND SOLUTIONS

Volumetric Solutions known also as Standard Solutions are solutions of reagents of known concentration intended primarily for use in quantitative determinations. Concentrations are usually expressed in terms of normality or molarity.

Normal Solutions

Normal solutions are solutions that contain 1 gram equivalent weight of the active substance in each 1000 ml of solution, that is, an amount equivalent to 1.0079 g of hydrogen or 7.9997 g of oxygen. Normal solutions and solutions bearing a specific relationship to normal solutions and used in volumetric determinations, are designated as follows: normal, *N*; double-normal, *2N*; half-normal, *0.5N*; tenth-normal, *0.1N* and so on.

Molar Solutions

Molar solutions are solutions that contain, in 1000 ml

1 gram-molecule of the reagent. Thus, each litre of a molar solution of sodium nitrite contains 69.0 g of $NaNO_2$ and each molar solution of disodium ethylenediaminetetraacetate (disodium edetate) contains 372.2 g of $C_{10}H_{14}N_2Na_2O_8,2H_2O$. Solutions containing, in 1000 ml, one-tenth of a gram-molecule of the reagent are designated 'tenth-molar', *0.1M*, and other molarities are similarly indicated.

Preparation and Standardisation of Volumetric Solutions

It is not always possible to prepare volumetric solutions of a desired theoretical normality or molarity, and it is not essential. A solution of approximately the desired normality or molarity is prepared and standardised by titration against a solution of a primary standard. The normality or molarity factor so obtained is used in all calculations where such standardised solutions are employed. As the strength of a standard solution may change upon standing, the normality or molarity factor should be redetermined frequently. Volumetric solutions should not differ from the prescribed strength by more than 10 per cent.

When solutions of a reagent are used in several normalities or molarities, the details of the preparation and standardisation are usually given for the most commonly used strength. Stronger or weaker solutions are prepared and standardised using proportionate amounts of the reagents or by making an exact dilution of a stronger solution. Volumetric solutions prepared by dilution should be restandardised either as directed for the stronger solution or by comparison with another volumetric solution having a known ratio to the stronger solution.

The water used in preparing volumetric solutions complies with the requirements of the monograph on Purified Water. When used for the preparation of unstable solutions such as potassium permanganate or sodium thiosulphate, it should be freshly boiled and cooled. When a solution is to be used in an assay in which the end-point is determined by an electrochemical process (e.g. potentiometrically), the solution must be standardised in the same way.

Blank Determinations

Where a monograph directs that "any necessary correction" be made by a blank determination, the determination should be done using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under test, but with the substance itself omitted.

Primary Standards

These are materials, which, after drying under the specified conditions, are recommended for use as primary standards in the standardisation of volumetric solutions. In the methods of preparation and standardisation of volumetric solutions given below, primary standards are designated by appending the letter P.S. to the name of the reagent. The following are recommended for use as primary standards:

Arsenic Trioxide – As_2O_3

Sublime *arsenic trioxide* in an appropriate apparatus and store over *silica gel*.

Benzoic Acid – $C_7H_6O_2$

Sublime *benzoic acid* in an appropriate apparatus and store in a tightly-closed container.

Potassium Bromate – $KBrO_3$

Recrystallise *potassium bromate* from boiling water. Collect the crystals and dry to constant weight at 130°. Store in tightly-closed containers.

Potassium Dichromate – $K_2Cr_2O_7$

Heat *potassium dichromate* to 140° to 150° in an oven, cool in a desiccator and powder in a glass mortar.

Potassium Hydrogen Phthalate – $C_8H_5KO_4$

Recrystallise *potassium hydrogen phthalate* from boiling *water*, collect the crystals at a temperature above 35° and dry to constant weight at 110°. Store in tightly-closed containers.

Potassium Iodate – KIO_3

Recrystallise *potassium iodate* from boiling *water*. Collect the crystals and dry to constant weight at 120°. Store in tightly-closed containers.

Sodium Carbonate, Anhydrous – Na_2CO_3

Filter at room temperature a saturated solution of *sodium carbonate*. Introduce slowly into the filtrate a stream of *carbon dioxide* with constant cooling and stirring. After about two hours collect the precipitate on a sintered-glass filter. Wash the filter with ice-cold *water* containing *carbon dioxide*. After drying at 100° to 105°, heat to constant weight at 270° to 300°, stirring from time to time. Store in tightly-closed containers.

Sodium Chloride – NaCl

To one volume of a saturated solution of *sodium chloride* add two volumes of *hydrochloric acid*. Collect the crystals formed and wash with *hydrochloric acid*. Remove the hydrochloric acid by heating on a water-bath and dry the crystals to constant weight at 300°. Store in tightly-closed containers.

Sulphanilic Acid – $C_6H_7NO_3S$

Recrystallise *sulphanilic acid* from boiling *water*. Filter and dry to constant weight at 100° to 105°.

Zinc, Granulated – Zn

Wash *granulated zinc* with *dilute hydrochloric acid*, followed by *water, alcohol* and finally *acetone*. Dry at 100° for five minutes and cool in a desiccator over *silica gel*.

Preparation and Standardisation

Although only one method of standardisation is given for each volumetric solution, other methods of standardisation, capable of yielding at least the same degree of accuracy, may be used. For those salts that usually are available as primary standards or that are available as highly purified salts of primary standard quality, volumetric solutions may be prepared by accurately weighing a suitable quantity of the reagent and dissolving it to produce a specific volume of solution of known concentration.

Wherever practicable, all volumetric solutions should be prepared and standardised at 25°. If a titration is carried out at markedly different temperature, the volumetric solution should be standardised at the same temperature or a suitable temperature correction should be made.

Ammonium Thiocyanate, 0.1 N : NH_4SCN = 76.12; 7.612 g in 1000 ml

Dissolve about 8 g of *ammonium thiocyanate* in 1000 ml of *water*, and standardise the solution as follows:

Pipette 30 ml of standardised *0.1 N silver nitrate* into a glass-stoppered flask, dilute with 50 ml of *water*, then add 2 ml of *nitric acid* and 2 ml of *ferric ammonium sulphate solution*, and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown colour. Each ml of *0.1 N silver nitrate* is equivalent to 0.007612 g of NH_4SCN.

Barium Hydroxide, 0.1 N : $Ba(OH)_2,8H_2O$ = 315.47; 15.77 g in 1000 ml

Dissolve 15.8 g of *barium hydroxide* in sufficient *carbon dioxide-free water* to produce 1000 ml and standardise the solution as follows:

Titrate, in an atmosphere of nitrogen, 50.0 ml of the solution with standardised *0.1 N hydrochloric acid*, using *phenolphthalein solution* as indicator. Each ml of *0.1 N hydrochloric acid* is equivalent to 0.01577 g of $Ba(OH)_2,8H_2O$.

Barium Perchlorate, 0.01 M : $Ba(ClO_4)_2$ = 336.27

Dissolve 3.5 g of *barium hydroxide* in a mixture of 15 ml of *water* and 1.5 ml of *perchloric acid*, adjust to pH 3 with *perchloric acid* and filter if necessary. To the solution add 150 ml of *alcohol* and dilute with *water* to 250 ml; dilute the solution further with *acetate buffer pH 3.7* to 1000 ml. Standardise the solution as follows:

Pipette 25 ml of standardised *0.02 N sulphuric acid* into a flask, add 50 ml of *acetate buffer pH 3.7* and titrate with the barium perchlorate solution, using 0.5 ml of *alizarin red S solution* as indicator, to an orange-red end-point. Each ml of *0.02 N sulphuric acid* is equivalent to 0.003362 g of $Ba(ClO_4)_2$.

Benzethonium Chloride, 0.004 M : $C_{27}H_{42}ClNO_2,H_2O$ = 466.12

Weigh accurately 1.792 g of *benzethonium chloride*, previously dried to constant weight at 100° to 105°, and dissolve in sufficient *water* to produce 1000.0 ml and standardise the solution as follows:

Pipette 20 ml of *0.004 M sodium lauryl sulphate*, 10 ml of *water*, 15 ml of *chloroform*, 10 ml of *dilute sulphuric acid*, and 1 ml of *dimethyl yellow-oracet blue B solution*, and titrate with the benzethonium chloride solution until the chloroform becomes green. Calculate the molarity.

Bromine, 0.1 N : Br = 79.90;·7.99 g in 1000 ml

Dissolve 3 g of *potassium bromate* and 15 g of *potassium bromide* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Pipette 25 ml of the solution into a 500-ml iodine flask, and dilute with 120 ml of *water*. Add 5 ml of *hydrochloric acid*, insert the stopper in the flask, and shake it gently. Then add 5 ml of *potassium iodide solution*, again insert the stopper, allow it to stand for five minutes in the dark. Titrate the liberated iodine with standardised *0.1 N sodium thiosulphate*, adding 3 ml of *starch solution* towards the end-point. Each ml of *0.1 N sodium thiosulphate* is equivalent to 0.01598 g of Br_2.

NOTE – Store 0.1 N bromine in dark amber-coloured, glass-stoppered bottles.

Ceric Ammonium Sulphate, 0.1 N (Ammonium Ceric Sulphate, 0.1 N) : $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$ = 632.53; 63.26 g in 1000 ml

Dissolve 66 g of *ceric ammonium sulphate*, with the aid of gentle heat, in a mixture of 30 ml of *sulphuric acid* and 500 ml of *water*. Cool, filter the solution, if turbid, and dilute with *water* to 1000 ml. Standardise the solution as follows:

Weigh accurately about 0.2 g of *arsenic trioxide P.S.*, previously dried at 105° for one hour, and transfer to a 500-ml conical flask. Wash down the inner walls of the flask with 25 ml of a 8.0 per cent w/v solution of *sodium hydroxide*, swirl to dissolve, add 100 ml of *water* and mix. Add 30 ml of *dilute sulphuric acid*, 0.15 ml of *osmic acid solution*, 0.1 ml of *ferroin sulphate solution* and slowly titrate with the ceric ammonium sulphate solution until the pink colour is changed to a very pale blue. Each 4.946 mg of *arsenic trioxide P.S.* is equivalent to 1 ml of *0.1 N ceric ammonium sulphate* or 0.06326 g of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

2,6-Dichlorophenolindophenol Solution, Standard

To 50 mg of *2,6-dichlorophenolindophenol sodium*, previously stored in a desiccator over *soda lime*, add 50 ml of *water* containing 42 mg of *sodium bicarbonate*, shake vigorously, and when the dye is dissolved, add *water* to produce 200 ml. Filter into an amber-coloured bottle. Standardise the solution as follows:

Weigh accurately about 50 mg of *ascorbic acid R.S.* and transfer to a glass-stoppered, 50-ml volumetric flask with the aid of a sufficient volume of *metaphosphoric-acetic acids solution* to produce 50 ml. Immediately transfer 2.0 ml of the solution to a 50-ml conical flask containing 5 ml of *metaphosphoric-acetic acids solution*, and titrate rapidly with the dichlorophenolindophenol solution until a distinct rose-pink colour persists for at least five seconds. Perform a blank titration by titrating 7 ml of *metaphosphoric-acetic acids solution* plus a volume of *water* equal to the volume of the dichlorophenolindophenol solution used in titrating the ascorbic acid solution. Express the concentration of the standard solution in terms of its equivalent in mg of ascorbic acid.

Standard 2,6-Dichlorophenolindophenol Solution must not be used later than 3 days after preparation, and must be standardised immediately before use.

Disodium Ethylenediaminetetraacetate, 0.05 M (Disodium Edetate, 0.05 M) : $C_{10}H_{14}N_2Na_2O_8,2H_2O$ = 372.24; 18.61 g in 1000 ml

Dissolve 18.6 g of *disodium ethylenediaminetetraacetate* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.8 g of *granulated zinc P.S.*, dissolve by gentle warming in 12 ml of *dilute hydrochloric acid* and five drops of *bromine water*. Boil to remove excess bromine, cool, and add sufficient *water* to produce 200.0 ml. Pipette 20 ml of the resulting solution into a flask and nearly neutralise with *2N sodium hydroxide*. Dilute to about 150 ml with *water*, add sufficient *ammonia buffer pH 10* to dissolve the precipitate and add 5 ml in excess. Add 50 mg of *mordant black 11 mixture* and titrate with the disodium ethylenediaminetetraacetate solution until the solution turns green. Each 0.003269 g of *granulated zinc* is equivalent to 1 ml of *0.05M disodium ethylenediaminetetraacetate*.

Ferric Ammonium Sulphate, 0.1 N

$FeNH_4(SO_4)_2,12H_2O$ = 482.18; 48.22 g in 1000 ml.

Dissolve 50 g of *ferric ammonium sulphate* in a mixture of 300 ml of *water* and 6 ml of *sulphuric acid*, dilute with *water* to 1000 ml, and mix. Standardise the solution as follows:

Measure accurately about 30 ml of the solution into a glass-stoppered flask, add 5 ml of *hydrochloric acid*, mix, and add a solution of 3 g of *potassium iodide* in 10 ml of *water*. Insert the stopper, allow to stand for ten minutes in the dark, then titrate the liberated iodine with standardised *0.1N sodium thiosulphate*, adding 3 ml of *starch solution* as the end-point is approached. Perform a blank determination and make any necessary correction. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.04822 g of $FeNH_4(SO_4)_2,12H_2O$.

NOTE – Store 0.1N ferric ammonium sulphate in tightly-closed, light-resistant containers.

Ferrous Ammonium Sulphate, 0.1 N

$Fe(NH_4)_2(SO_4)_2,6H_2O$ = 392.13; 39.21 g in 1000 ml.

Dissolve 40 g of *ferrous ammonium sulphate* in a previously cooled mixture of 40 ml of *sulphuric acid* and 200 ml of *water*, dilute with *water* to 1000 ml, and mix. Standardise the solution as follows:

Measure accurately 25 ml of the solution into a flask, add two drops of *orthophenanthroline solution* and titrate with standardised *0.1N ceric ammonium sulphate* until the red colour is changed to pale blue. Each ml of *0.1N ceric ammonium sulphate* is equivalent to 0.03921 g of $Fe(NH_4)_2(SO_4)_2,6H_2O$.

Hydrochloric Acid, N ; HCl = 36.46; 36.46 g in 1000 ml

Dilute 85 ml of *hydrochloric acid* with *water* to 1000 ml and standardise the solution as follows:

Weigh accurately about 1.5 g of *anhydrous sodium carbonate P.S.*, previously heated at about 270° for one hour. Dissolve it in 100 ml of *water* and add two drops of *methyl red solution*. Add the acid slowly from a burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration. Heat again to boiling, and titrate further as necessary until the faint pink colour is no longer affected by continued boiling. Each 0.05299 g of *anhydrous sodium carbonate* is equivalent to 1 ml of *N hydrochloric acid*.

Iodine, 0.1 N : I = 126.90; 12.69 g in 1000 ml

Dissolve about 14 g of *iodine* in a solution of 36 g of *potassium iodide* in 100 ml of *water*, add three drops of *hydrochloric acid*, dilute with *water* to 1000 ml and standardise the solution as follows:

Weigh accurately about 0.15 g of *arsenic trioxide P.S.*, previously dried at 105° for one hour, and dissolve in 20 ml of *N sodium hydroxide* by warming, if necessary. Dilute with 40 ml of *water*, add two drops of *methyl orange solution* and follow with *dilute hydrochloric acid* until the yellow colour is changed to pink. Then add 2 g of *sodium bicarbonate*, dilute with 50 ml of *water*, and add 3 ml of *starch solution*. Slowly add the iodine solution from a burette until a permanent blue colour is produced. Each 0.004946 g of *arsenic trioxide* is equivalent to 1 ml of *0.1N iodine*.

NOTE – Store 0.1N Iodine in amber-coloured, glass-stoppered bottles.

Lead Nitrate, 0.05 M : $Pb(NO_3)_2$ = 331.2; 16.56 g in 1000 ml

Dissolve 16.5 g of *lead nitrate* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Pipette 50 ml of the solution into a flask, add 50 mg of *xylenol orange mixture* and sufficient *hexamine* to produce a violet-pink colour, and titrate with standardised *0.1M disodium ethylenediaminetetraacetate* to a lemon-yellow end-point. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.03312 g of $Pb(NO_3)_2$.

Lithium Methoxide, 0.1 N : CH_3OLi = 37.98; 3.798 g in 1000 ml

Dissolve in small portions 0.7 g of freshly cut *lithium* in 150 ml of *methyl alcohol*, cooling the flask during addition of the metal. When reaction is complete add 850 ml of *toluene*. If cloudiness or precipitation occurs, add sufficient *methyl alcohol* to clarify the solution. Store the solution in a container suitably protected from carbon dioxide and moisture. Standardise the solution as follows:

Weigh accurately about 0.25 g of *benzoic acid P.*, dissolve in 25 ml of *dimethylformamide* and titrate with the lithium methoxide solution, using *quinaldine red solution* as indicator and protecting the solution from carbon dioxide of the atmosphere. Correct for the volume of the lithium methoxide solution consumed by 25 ml of *dimethylformamide*. Each 0.01221 g of *benzoic acid* is equivalent to 1 ml of *0.1N lithium methoxide*.

NOTE – 0.1N Lithium Methoxide must be standardised immediately before use.

Magnesium Sulphate, 0.05 M : $MgSO_4,7H_2O$ = 246.47; 12.325 g in 1000 ml

Dissolve 12.5 g of *magnesium sulphate* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Pipette 30 ml of the solution into a flask, add 20 ml of *water*, 10 ml of *strong ammonia-ammonium chloride solution* and 50 mg of *mordant black 11 mixture*. Titrate with standardised *0.1M disodium ethylenediaminetetraacetate* until the colour changes from violet to full blue. Each ml of *0.1M disodium ethylenediaminetetraacetate* is equivalent to 0.02465 g of $MgSO_4,7H_2O$.

Mercuric Acetate, 0.05 M : $(CH_3CO_2)_2Hg$ = 318.68; 15.93 g in 1000 ml

Dissolve 16 g in 5 ml of *glacial acetic acid* and sufficient *water* to produce 1000 ml and standardise the solution as follows:

To 25.0 ml of the solution add 0.1 g of *xylenol orange mixture* and 2 g of *hexamine*, and titrate with standardised *0.05M disodium ethylenediaminetetraacetate* to a yellow end-point. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.01593 g of $C_4H_6HgO_4$.

Oxalic Acid, 0.1 N : $H_2C_2O_4,2H_2O$ = 126.07; 6.303 g in 1000 ml.

Dissolve 6.45 g of *oxalic acid* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Pipette 30 ml of the solution into a beaker, add 150 ml of *water*, 7 ml of *sulphuric acid* and heat to about 70°. Add slowly from a burette freshly standardised *0.1N potassium permanganate* with constant stirring, until a pale-pink colour, which persists for fifteen seconds, is produced. The temperature at the conclusion of the titration should not be less than 60°. Each ml of *0.1N potassium permanganate* is equivalent to 0.006303 g of $H_2C_2O_4,2H_2O$.

Perchloric Acid, 0.1 N (in Glacial Acetic Acid) : $HClO_4$ = 100.46; 10.05 g in 1000 ml

NOTE – In the tests and assays of the pharmacopoeia, this solution is specified as "0.1N perchloric acid". Thus the solution in glacial acetic acid is to be used, unless the words "in dioxan" are stated.

Mix 8.5 ml of *perchloric acid* with 500 ml of *glacial acetic acid* and 21 ml of *acetic anhydride*, cool, and add *glacial acetic acid* to make 100 ml. Alternatively, mix 11 ml of *perchloric acid (60 per cent)* with 500 ml of *glacial acetic acid* and 30 ml of *acetic anhydride*, cool, and add glacial acetic acid to make 1000 ml.

Allow the prepared solution to stand for one day for the excess acetic anhydride to be combined, and carry out the *determination of water*, Appendix 3.3.25. If the water content exceeds 0.05 per cent, add more *acetic anhydride*. If the solution contains no titratable water, add sufficient *water* to obtain a content of between 0.02 per cent and 0.05 per cent of water. Allow the solution to stand for one day, and again titrate the water content. The solution so obtained should contain between 0.02 per cent and 0.05 per cent of water. Standardise the solution as follows:

Weigh accurately about 0.7 g of *potassium hydrogen phthalate P.S.*, previously powdered lightly and dried at 120° for two hours, and dissolve it in 50 ml of *glacial acetic acid*. Add two drops of *crystal-violet solution* and titrate with the perchloric acid solution until the violet colour changes to emerald-green. Deduct the volume of the perchloric acid consumed by 50 ml of the glacial acetic acid. Each 0.02042 g of *potassium hydrogen phthalate* is equivalent to 1 ml of *0.1N perchloric acid*.

NOTE – Other strengths of perchloric acid should be prepared by diluting 0.1N perchloric acid appropriately with glacial acetic acid.

Perchloric Acid in Dioxan, 0.1 N : $HClO_4$ = 100.46; 10.05 g in 1000 ml

Mix 8.5 ml of *perchloric acid* with sufficient *dioxan*, which has been specially purified by adsorption, to make 1000 ml. Standardise the solution as described under *0.1N perchloric acid*.

Potassium Bromate, 0.1 N : $KBrO_3$ = 167.00; 2.784 g in 1000 ml

Dissolve 2.784 g of *potassium bromate* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Transfer an accurately measured volume of about 30 ml of the solution to a glass-stoppered flask, add 3 g of *potassium iodide*, followed by 3 ml of *hydrochloric acid*. Allow to stand for five minutes, then titrate the liberated iodine with standardised *0.1N sodium thiosulphate*, adding 3 ml of *starch solution* as the end-point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.002784 g of $KBrO_3$.

Potassium Bromide-Bromate, 0.1 N

Dissolve 2.78 g of *potassium bromate* and 12.0 g of *potassium bromide* in *water* and dilute with *water* to 1000 ml. Standardise this solution as described under *0.1N potassium bromate*.

Potassium Dichromate, 0.1 N : $K_2Cr_2O_7$ = 294.18; 4.903 g in 1000 ml

Weigh accurately 4.903 g of *potassium dichromate P.S.*, previously powdered and dried at 20° for four hours, and dissolve in sufficient *water* to produce 1000 ml.

Potassium Hydroxide, Alcoholic, 0.1 N : KOH = 56.11; 5.611 g in 1000 ml

Dissolve about 7 g of *potassium hydroxide* in 5 ml of *water* and add sufficient *aldehyde-free alcohol* to produce 1000 ml. Allow the solution to stand in a tightly-stoppered bottle for twenty-four hours. Then quickly decant the clear supernatant liquid into a suitable, tightly-closed container, and standardise the solution as follows:

Pipette 25 ml of standardised *0.1N hydrochloric acid* into a flask, dilute with 50 ml of *water*, add two drops of *phenolphthalein solution* and titrate with the alcoholic potassium hydroxide solution until a permanent pale-pink colour is produced. Each ml of *0.1N hydrochloric* acid is equivalent to 0.005611 g of KOH.

NOTE – Store in tightly-stoppered bottles and protect from light.

Potassium Hydroxide in Alcohol (60 per cent), 0.5 N : KOH = 56.11; 28.05 g in 1000 ml

Dissolve 28.05 g of *potassium hydroxide* in sufficient *alcohol (60 per cent)* to produce 1000 ml and standardise the solution as follows:

Pipette 25 ml of standardised *0.5N hydrochloric acid* into a flask, add a few drops of a 0.2 per cent w/v solution of *methyl orange* in *alcohol (60 per cent)* and titrate with the alcoholic potassium hydroxide solution until a full yellow colour is produced. Each ml of *0.5N hydrochloric* acid is equivalent to 0.02806 g of KOH.

Potassium Hydroxide in Alcohol (90 per cent), N KOH = 56.11; 56.11 g in 1000 ml

Dissolve 60 g of *potassium hydroxide* in sufficient *alcohol (90 per cent)* to produce 1000 ml, allow the solution to stand for twenty-four hours, decant the clear solution, and ascertain its exact strength by the method described under *0.1N alcoholic potassium hydroxide*.

Potassium Iodate, 0.05 M : KIO_3 = 214.00; 10.70 g in 1000 ml

Weigh accurately 10.700 g of *potassium iodate P.S.*, previously dried at 110° to constant weight, in sufficient *water* to produce 1000 ml.

Potassium Permanganate, 0.1 N : $KMnO_4$ = 158.03; 3.161 g in 1000 ml

Dissolve about 3.3 g of *potassium permanganate* in 1000 ml of *water*, heat on a water-bath for one hour, and allow to stand for two days. Filter through glass wool and standardise the solution as follows:

To an accurately measured volume of about 25 ml of the solution in a glass-stoppered flask add 2 g of *potassium iodide*, followed by 10 ml of *N sulphuric acid*. Titrate the liberated iodine with standardised *0.1N sodium thiosulphate*, adding 3 ml of *starch solution* as the end-point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of *0.1N sodium thiosulphate* is equivalent to 0.003161 g of $KMnO_4$.

Silver Nitrate, 0.1 N : $AgNO_3$ = 169.87; 16.99 g in 1000 ml

Dissolve about 17 g in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.1 g of *sodium chloride P.S.*, previously dried at 110° for two hours and dissolve in 5 ml of *water*. Add 5 ml of *acetic acid*, 50 ml of *methyl alcohol* and three drops of *eosin solution*. Stir, preferably with magnetic stirrer, and titrate with the silver nitrate solution. Each 0.005844 g of *sodium chloride* is equivalent to 1 ml of *0.1N silver nitrate*.

Sodium Arsenite, 0.2 N : $NaAsO_2$ = 129.91; 12.99 g in 1000 ml

Weigh accurately 9.891 g of *arsenic trioxide P.S.*, previously dried at 105° for one hour, in a mixture of 20 ml of *5N sodium hydroxide* and 40 ml of *water*, dilute to 800 ml with *water*, and add *2N hydrochloric acid* until the so-

lution is neutral. Dissolve 4 g of *sodium bicarbonate* in the solution and dilute with *water* to 1000 ml.

Sodium Hydroxide, N : NaOH = 40.00; 40.00 g in 1000 ml.

Dissolve 162 g of *sodium hydroxide* in 150 ml of *carbon dioxide-free water*, cool the solution to room temperature, and filter through hardened filter paper. Transfer 54.5 ml of the clear filtrate to a tightly-closed polythene container, and dilute with *carbon dioxide-free water* to 1000 ml. Standardise the solution as follows:

Weigh accurately about 5 g of *potassium hydrogen phthalate P.S.*, previously powdered and dried at 120° for two hours, and dissolve in 75 ml of *carbon dioxide-free water*. Add two drops of *phenolphthalein solution* and titrate with the sodium hydroxide solution until a permanent pink colour is produced. Each 0.2042 g of *potassium hydrogen phthalate* is equivalent to 1 ml of *N sodium hydroxide*.

NOTES — (1) Store N sodium hydroxide in bottles with well-fitted suitable stoppers which prevent access to atmospheric carbon dioxide. (2) Solutions of lower concentration are prepared by quantitatively diluting accurately measured volumes of N sodium hydroxide with sufficient carbon dioxide-free water to give the desired concentration. (3) Volumetric solutions of sodium hydroxide must be restandardised frequently.

Sodium Hydroxide, Alcoholic, 0.1 N : NaOH = 40.00; 4.0 g in 1000 ml

Dissolve 4.2 g of *sodium hydroxide* in 5 ml of *water* and add sufficient *aldehyde-free alcohol* to produce 1000 ml. Allow the solution to stand in a tightly-stoppered bottle for twenty-four hours. Then quickly decant the clear supernatant liquid into a suitable, tightly-closed container. Standardise the solution as follows:

Weigh accurately about 0.6 g of *benzoic acid P.S.*, dissolve in a mixture of 30 ml of *alcohol* and 6 ml of *water* and titrate with the alcoholic sodium hydroxide solution, using *thymolphthalein solution* as indicator. Each 0.01221 g of *benzoic acid* is equivalent to 1 ml of *0.1N alcoholic sodium hydroxide*.

NOTE — Store in tightly-stoppered bottles and protect from light.

Sodium Hydroxide (Carbonate-free), 0.02 N : NaOH = 40.00; 0.80 g in 1000 ml

Dissolve *sodium hydroxide* in *water* to produce a 40 to 60 per cent w/v solution and allow to stand. Taking precautions to avoid absorption of carbon dioxide, siphon off the clear supernatant liquid and dilute with *carbon dioxide-free water* to give approximately the required strength. The solution complies with the following test:

Titrate 45 ml of hydrochloric acid of the same normality as the solution under test with the sodium hydroxide solution, using *phenolphthalein solution* as indicator. At the end-point, add just sufficient of the acid to discharge the pink colour and boil to reduce the volume to 20 ml. Add, whilst boiling, sufficient of the acid again to discharge the pink colour which does not reappear on continued boiling; not more than 0.1 ml of the acid is required.

Standardise the sodium hydroxide solution as directed under *N sodium hydroxide*.

Sodium Lauryl Sulphate, 0.004 M

Dissolve 1.1530 g of *purified sodium lauryl sulphate*, calculated with reference to the substance dried at 105° for two hours, in sufficient *water* to produce 1000 ml.

0.004 M Sodium Lauryl Sulphate should be freshly prepared.

Sodium Methoxide, 0.1 N : CH_3ONa = 54.02; 5.402 g in 1000 ml

Cool in ice-water 150 ml of *methyl alcohol* and add, in small portions, about 2.5 g of freshly cut *sodium*. When the metal has dissolved, add sufficient *toluene*, previously dried over sodium wire, to produce 1000 ml. Store preferably in a container protected from carbon dioxide and moisture. Standardise the solution as follows immediately before use:

Weigh accurately about 0.4 g of *benzoic acid P.S.*, dissolve in 80 ml of *dimethylformamide*, add three drops of *thymolphthalein solution* and titrate with the sodium methoxide solution to a blue end-point. Correct for the volume of the sodium methoxide solution consumed by 80 ml of the dimethylformamide. Each 0.01221 g of *benzoic acid* is equivalent to 1 ml of *0.1N sodium methoxide*.

Sodium Nitrite, 0.1 M : $NaNO_2$ = 69.00; 6.900 g in 1000 ml.

Dissolve 7.5 g of *sodium nitrite* in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.5 g of *sulphanilamide P.S.*, previously dried at 105° for three hours, and transfer to a suitable beaker. Add 20 ml of *hydrochloric acid* and 50 ml of *water*, stir until dissolved, and cool to 15°. Carry out the *nitrite titration*, Appendix 3.3.4. Each 0.01722 g of *sulphanilamide* is equivalent to 1 ml of *0.1M sodium nitrite*.

Sodium Thiosulphate, 0.1 N : $Na_2S_2O_3,5H_2O$ = 248.17; 24.82 g in 1000 ml

Dissolve about 26 g of *sodium thiosulphate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000 ml with the same solvent. Standardise the solution as follows:

Dissolve 0.200 g of *potassium bromate P.S.* in sufficient *water* to produce 250 ml. To 50 ml of this solution, add 2 g of *potassium iodide* and 3 ml of *2N hydrochloric*

acid and titrate with the sodium thiosulphate solution using *starch solution*, added towards the end of the titration, as indicator until the blue colour is discharged. Each 0.002784 g of *potassium bromate* is equivalent to 1 ml of *0.1N sodium thiosulphate*.

NOTE – Restandardise 0.1N sodium thiosulphate frequently.

Sulphuric Acid, N : H_2SO_4 = 98.07; 49.04 g in 1000 ml

Add slowly, with stirring, 30 ml of *sulphuric acid* to about 1020 ml of *water*, allow to cool to 25°, and standardise against *anhydrous sodium carbonate P.S.* as described under *N hydrochloric acid*. Each 0.05299 g of *anhydrous sodium carbonate* is equivalent to 1 ml of *N sulphuric acid*.

Tetrabutylammonium Hydroxide, 0.1 N
$(C_4H_9)_4$ NOH = 259.48; 25.95 g in 1000 ml.

Dissolve 40 g of *tetrabutylammonium iodide* in 90 ml of *dehydrated methyl alcohol* in a glass-stoppered flask. Place in an ice-bath, add 20 g of powdered *silver oxide*, insert the stopper in the flask and agitate vigorously for one hour. Centrifuge a few ml, and test the supernatant liquid for *iodide*, Appendix 3.1. If the test is positive, add an additional 2 g of *silver oxide*, and continue to allow to stand for thirty minutes with intermittent agitation. When all of the iodide has reacted, filter through a fine sintered-glass filter. Rinse the flask and the filter with three quantities, each of 50 ml, of *anhydrous toluene*. Add the washings to the filtrate and dilute to 1000 ml with *anhydrous toluene*. Flush the solution for ten minutes with dry, carbon dioxide-free *nitrogen*. Store in a container protected from carbon dioxide and moisture, and discard after 60 days.

Alternatively, prepare the solution by diluting a suitable volume of commercially available tetrabutylammonium hydroxide solution in *methyl alcohol* with a mixture of four volumes of *anhydrous toluene* and 1 volume of *dehydrated methyl alcohol*. Standardise the solution on the day of use as follows:

Weigh accurately about 0.4 g of *benzoic acid P.S.*, dissolve in 80 ml of *dimethylformamide*, add three drops of a 1 per cent w/v solution of *thymol blue* in *dimethylformamide* and titrate with the tetrabutylammonium hydroxide solution to a blue end-point. The solution must be protected from carbon dioxide of the atmosphere throughout the titration. Perform a blank determination and make any necessary correction. Each 0.01221 g of *benzoic acid* is equivalent to 1 ml of *0.1N tetrabutylammonium hydroxide*.

Tetrabutylammonium Hydroxide in Isopropyl Alcohol, 0.1 N

Prepare as directed under *0.1N tetrabutylammonium hydroxide* but use *isopropyl alcohol* instead of *anhydrous toluene*. Standardise the solution as directed under *0.1N tetrabutylammonium hydroxide*.

Tetrabutylammonium Hydroxide in Methyl Alcohol, 0.1 N

Prepare as directed under *0.1N tetrabutylammonium hydroxide* but use *methyl alcohol* instead of *anhydrous toluene*. Standardise the solution as directed under *0.1N tetrabutylammonium hydroxide*.

Titanous Chloride, 0.1 N : $TiCl_3$ = 154.26; 15.43 g in 1000 ml

Add 103 ml of *titanous chloride solution* to 100 ml of *hydrochloric acid*, dilute to 1000 ml with recently boiled and cooled *water*, and mix. Standardise, immediately before use, as follows:

Place an accurately measured volume of about 30 ml of standardised *0.1N ferric ammonium sulphate* in a flask and pass in a rapid stream of *carbon dioxide* until all the air has been removed. Add the titanous chloride solution from a burette and in an atmosphere of *carbon dioxide* until near the calculated end-point, then add 5 ml of *ammonium thiocyanate solution*, and continue the titration until the solution is colourless. Each ml of *0.1N ferric ammonium sulphate* is equivalent to 0.01543 g of $TiCl_3$.

Zinc Chloride, 0.05 M : $ZnCl_2$ = 136.29; 6.815 g in 1000 ml

Dissolve 3.3 g of *granulated zinc*, previously washed with *0.1N hydrochloric acid* and then with *water*, in the minimum amount of *2N hydrochloric acid* and add sufficient *water* to produce 1000 ml. Standardise the solution as follows:

To 25 ml of the solution add 4 g of *ammonium acetate* and 25 ml of *water*. Add 50 mg of *xylenol orange mixture* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.05M disodium ethylenediaminetetraacetate* until the colour changes to yellow. Each ml of *0.05M disodium ethylenediaminetetraacetate* is equivalent to 0.006815 g of $ZnCl_2$.

APPENDIX 8

8. Reference Substances

Reference Substances are specifically required in many pharmacopoeial tests and assays. They are substances selected for their high purity, critical characteristics, and suitability for the intended purpose. They are not intended for use as drugs.

Reference Substances are maintained and distributed by the Central Drug Laboratory, Calcutta.

Where the letters R.S. appear after the name of a substance in a test or assay in the individual monograph or in an Appendix, the relevent I.P. Reference Substance must be used.

In order to serve the intended purpose, it is important that each I.P. Reference Substance is properly stored, handled, and used. Reference Substances should be stored in a dry place, away from heat and protected from light. Special storage conditions, where necessary, are usually given on the label.

9. Tables

9.1 NAMES, SYMBOLS, AND ATOMIC WEIGHTS OF ELEMENTS

$^{12}C = 12$

Element	Symbol	Atomic Weight	Element	Symbol	Atomic Weight
Aluminium	Al	26.98154	Manganese	Mn	54.9380
Antimony	Sb	121.75	Mercury	Hg	200.59
Arsenic	As	74.9216	Molybdenum	Mo	95.94
Barium	Ba	137.33	Nitrogen	N	14.0067
Bismuth	Bi	208.9804	Oxygen	O	15.9994
Boron	B	10.81	Palladium	Pd	106.4
Bromine	Br	79.904	Phosphorus	P	30.97376
Cadmium	Cd	112.41	Platinum	Pt	195.09
Calcium	Ca	40.08	Potassium	K	39.0983
Carbon	C	12.011	Ruthenium	Ru	101.07
Cerium	Ce	140.12	Selenium	Se	78.96
Chlorine	Cl	35.453	Silicon	Si	28.0855
Chromium	Cr	51.996	Silver	Ag	107.868
Cobalt	Co	58.9332	Sodium	Na	22.98977
Copper	Cu	63.546	Strontium	Sr	87.62
Fluorine	F	18.998403	Sulphur	S	32.06
Gold	Au	196.9665	Technetium	Tc	(97)
Helium	He	4.00260	Thallium	Tl	204.37
Hydrogen	H	1.0079	Tin	Sn	118.69
Iodine	I	126.9045	Titanium	Ti	47.90
Indium	In	114.82	Tungsten	W	183.85
Iron	Fe	55.847	Uranium	U	238.029
Lanthanum	La	138.9055	Vanadium	V	50.9414
Lead	Pb	207.2	Xenon	Xe	131.30
Lithium	Li	6.941	Zinc	Zn	65.38
Magnesium	Mg	24.305	Zirconium	Zr	91.22

The atomic weights have been revised in accordance with the figures published by IUPAC (Atomic Weights of the Elements 1975, *Pure appl chem* 1976, **47**, 75).

9.2 WEIGHTS AND MEASURES

The names and symbols for units of measurement used by this Pharmacopoeia are generally the International System of Units (SI) or the CGS metric units.

The following multiplicative prefixes, which indicate decimal multiples and submultiples of the basic units are used:

Prefix	Symbol	Factor	Prefix	Symbol	Factor
mega	M	10^{6}	milli	m	10^{-3}
kilo	k	10^{3}	micro	μ	10^{-6}
deca	da	10^{1}	nano	n	10^{-9}
deci	d	10^{-1}	pico	p	10^{-12}
centi	c	10^{-2}			

The following basic units are employed in this Pharmacopoeia:

Quantity	Name of basic unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Amount of substance	mole	mol

Basic units, derived units*, multiples and sub-multiples that are employed are as follows:

Quantity	Unit	Symbol	Value in SI Units
Length	metre	m	–
	centimetre	cm	1 cm = 10^{-2}m
	millimetre	mm	1 mm = 10^{-3}m
	micrometre	μm	1 μm = 10^{-3} mm** = 10^{-6}m
	nanometre	nm**	1 nm = 10^{-3} μm = 10^{-6}mm = 10^{-9}m
Mass	kilogram	kg	–
	gram	g	1 g = 10^{-3} kg
	milligram	mg	1 mg = 10^{-3} g = 10^{-6} kg
	microgram	μg ***	1 μg = 10^{-3} mg = 10^{-6} g = 10^{-9} kg
	nanogram	ng	1 ng = 10^{-9} g
Volume	litre	l	1 l = 1 dm^3 = $10^{-3}m^3$
	millilitre	ml†	1 ml = 1 cm^3 = $10^{-6}m^3$
	microlitre	μl	1 μl = 10^{-3}ml = $10^{-9}m^3$

Quantity	Unit	Symbol	Value in SI Units
Time	hour	h	1 h = 60 min = 3600s
	minute	min	1 min = 60s
	second	s	–
	millisecond	ms	1 ms = 10^{-3}s
Temperature	degree celsius	°C	–
Electric current	ampere	A	–
	milliampere	mA	1 mA = 10^{-3}A
	nanoampere	nA	1 nA = 10^{-9}A
Electric potential	volt	V	–
	millivolt	mV	1 mV = 10^{-3}V
Electric resistance	ohm	Ω	–
Pressure	pascal	Pa	–
	kilopascal	kPa	1 kPa = 7.5 torr = 7.5 mm Hg

The following units of pressure (non-SI) are also used:

pound-force per square inch, psi; 1 psi ≈ 0.69 kPa millimetre of mercury, mm Hg, torr; 1 torr = 0.133 kPa

* Derived units are formed by combining the basic units according to certain algebraic relationships between the corresponding quantities

** Formerly the abbreviation mμ (for millimicron) was used.

*** Formerly the abbreviation mcg was used in the Pharmacopoeia; the symbol μg now is more widely accepted and this is used in this Pharmacopoeia.

† One millilitre (ml) is used as the equivalent of 1 cubic centimetre (cm^3; cc).

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